Characterization and prevention of *Fusarium* mycotoxicoses in the horse

Thesis

Characterization and Prevention of *Fusarium* Mycotoxicoses in the Horse:

A Thesis

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by

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ABSTRACT

Characterization and Prevention of *Fusarium* Mycotoxicoses in the Horse

Deoxynivalenol (DON), vomitoxin), zearalenone and fusaric acid are the most commonly found *Fusarium* mycotoxins in Ontario-grown feedstuffs. A survey was conducted to examine the degree of *Fusarium* mycotoxin and mold contamination of hay found on Ontario performance horse farms. Half of the hay sampled showed potentially significant levels of mycotoxins and mold contamination. The farm owner, trainer or manager's subjective opinion of the hay did not correlate to analysis results. The feeding of *Fusarium* mycotoxin-contaminated grains adversely affects the production of swine and poultry. Very little information is available, however, on adverse effects of feeding these mycotoxin-contaminated grains on the athletic or reproductive performance of horses. Trials were conducted, therefore, to determine the effects of feeding diets naturally-contaminated with *Fusarium* mycotoxins on horses. The contaminated diets were formulated by replacing corn and wheat of the control diet with grains naturally-contaminated with *Fusarium* mycotoxins. A polymeric glucomannan mycotoxin adsorbent (GM polymer) was also tested for its ability to prevent *Fusarium* mycotoxicoses. Trials examined both exercising and non-exercising horses. Feed intake of horses fed contaminated grains was reduced compared to controls in each trial. Supplementation of 0.2% GM polymer to the contaminated diet did not alter feed intake of exercising horses compared with those fed the unsupplemented contaminated diet. Supplementation of 0.2% GM polymer to the contaminated diet improved feed intake of non-exercising horses compared with those fed the unsupplemented contaminated diet. Serum activities of gamma-glutamyltransferase were higher (P = 0.047 and P = 0.027) in non-exercising horses fed the diet containing contaminated grain compared to those fed the control diet on d 7 and 14, but not on d 21 (P = 0.273). Supplementation of GM polymer to the

contaminated diet reduced ($P < 0.05$) serum gamma-glutamyltransferase activities of non-exercising horses compared with those fed unsupplemented contaminated diet on d 7 and 14. All hay was consumed regardless of concentrate fed. Weight loss from 0 to 21 d was observed in exercising horses fed contaminated grains as compared to controls ($P < 0.05$). No effect of diet was seen on variables used to measure athletic ability although the results showed an expected response to exercise for a fit horse. It was concluded that both non-exercising and exercising horses are susceptible to *Fusarium* mycotoxicoses as indicated by appetite suppression and weight loss.

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LIST OF ABBREVIATIONS

15-ADON	15-acetyl deoxynivalenol
AG	albumin to globulin
AP	alkaline phosphatase
AST	aspartate aminotransferase
BSTFA	*n,o*-bis[trimethylsily]triflouroacetamide
CFU	colony forming unit
CK	creatine kinase
D	day
DAS	diacetoxyscirpenal
DON	deoxynivalenol
ELEM	equine leukoencephalomalacia
ELISA	enzyme linked immunosorbent assay
FA	fusaric acid
GGT	gamma glutamyltransferase
GLDH	glutamate dehydrogenase
GM polymer	glucomannan polymer
Hb	haemoglobin
HCT	hematocrit
HPLC	high performance liquid chromatography
Ig	immunoglobulins
IgA	immunoglobulins A
IgG	immunoglobulins G
IgM	immunoglobulins M
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
Min	minute
NIV	nivalenol
PPE	porcine pulmonary edema
TMCS	trimethylchlorosilane

Published Material

Portions of the research reported in this thesis have been presented or published in:

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Raymond, S.L., T.K. Smith, and H.V.L.N. Swamy. 2003. Effects of feeding a blend of grains naturally-contaminated with Fusarium mycotoxins on feed intake, serum chemistry and hematology of horses. J. Anim. Sci. 81:2123-2130.

Raymond S.L., Heiskanen M.L., Smith T.K., Reiman M., Laitinen S. and A.F. Clarke. 2000. An investigation of the concentrations of selected Fusarium mycotoxins and the degree of mould contamination of Ontario field-dried hay. J. Equine Vet. Sci. 20: 616-621.

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Raymond, S.L., T.K. Smith, and H.V.L.N. Swamy. 2002. Effects of feeding a blend of grains naturally-contaminated with Fusarium mycotoxins on feed intake, serum chemistry and hematology of horses. J. Anim. Sci. 80(Suppl.1):296.

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TMS	trimethylsily
TMSI	*N*-trimethylsilyimidazole
WBC	white blood cells
ZEN	zearalenone

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CHAPTER 1

REVIEW OF LITERATURE

1.1. Molds and Production of Mycotoxins

The occurrence of mold and mycotoxins in food and animal feed is a problem of major concern internationally (Wood, 1992). Mycotoxin production can occur when conditions favourable to fungal growth occur on crops preharvest, at harvest, in storage or during the processing of feed (Palmgren and Lee, 1986). Mycotoxins are the products of secondary fungal metabolism although not all fungi produce mycotoxins. Environmental and nutritional parameters and growth stage of the mold govern toxin production. Molds that are present in the field before harvest can proliferate if moisture content remains high through inadequate processing and storage. Mold and subsequent mycotoxin contamination of a feedstuff or forage can increase in extreme environmental conditions such as drought, excessive precipitation, or sudden frost. It can also increase due to physical damage to a crop which allows fungal penetration of the plant tissue (Gregory, et al., 1963; Northolt, et al., 1976; Schindler, 1977). A large number of the common mycotoxins are produced by the *Fusarium* molds, which thrive in temperate climates worldwide and these mycotoxins are common contaminants of feed grains. A wet growing season followed by cool weather increases the likelihood that fungi, especially *Fusarium* and related mycotoxins, will be present in grains. High moisture content in grain encourages fungal growth while cool temperatures can increase the production of Fusarium mycotoxins. These fungi require a moisture content of 25% concurrent with a relative humidity of more than 90% in order to grow (Palmgren and Lee, 1986). Such conditions can commonly

occur when field-dried hay is not cured properly. Forage is the basis of most feeding programs for the horse, with long-stemmed, field dried hay being the traditional source.

1.2. Conditions favoring mycotoxin production

Aspergillus, Fusarium and *Penicillium* are the three most important genera of fungi that have been extensively studied in relation to mycotoxicoses. The large surface area and vigorous activity of fungal mycelia ensure a close relationship of the mold with the environment. Extrinsic factors such as temperature, pH, water activity, and chemical composition have an influence on growth and mycotoxin biosynthesis. Other factors such as plant-fungal interactions, interactions among microorganisms, and the presence of certain chemical agents can influence mold growth and mycotoxin synthesis (Smith and Henderson, 1991). In the field situation specific fungal species present may occur cyclically or seasonally. It is usually the case that the optimum conditions for mold growth do not coincide with those for mycotoxin production. The production of several different mycotoxins by the same species of fungi, or even the same strain, may not occur optimally under identical conditions. The production of deoxynivalenol (DON) and zearalenone (ZEN) by a single strain of *Fusarium graminearum,* for example, responded differently to changes in temperature. ZEN production reached a maximum at 25°C, while the production of DON continued to increase with increasing temperature, and at 28°C the production was more than twice that produced at 25°C (Greenhalgh et al., 1983). In field situations, DON and ZEN have been produced simultaneously in corn and wheat infected by the *Fusarium graminearum* (Cote et al., 1985).

1.3. Effects of Feed-Borne Mycotoxins on Horses

Until recently, the knowledge of the effects of mycotoxins on horses was generated through limited investigations with few numbers of animals or dependent on extrapolations using data

from other species (the pig or ruminant being the most common choices). A unique challenge is presented, however, when attempting to use non-equine data effectively since the horse is comparable to the ruminant in that it is a forage-grazing animal but has a gastrointestinal tract more closely related to a pig with the addition of a hind-gut fermentation process. The nature of the horse farm also makes the horse quite different from other livestock species. These other species are bred for growth, and meat yield and have a relatively short lifespan while in most cases the horse is bred for athletic performance, conformation, temperament, beauty and/or durability.

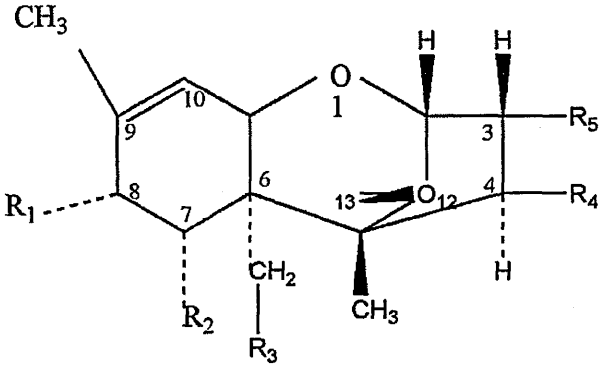
Threshold limiting values or safe concentrations of specific mycotoxins are, for the most part, unknown for the horse. Historically, mycotoxins were identified by their ability to produce severe, overt disease syndromes in animals as a result of acute exposure to mycotoxins. Chronic exposure, however, can elicit different pathologies. A concern is that for horses, especially elite athletes or breeding stock, exposure to low levels of mycotoxins may affect performance or breeding ability without the appearance of overt signs of disease. The outcome of chronic exposure may include general unthriftiness, suppression of the immune system and increased risk of secondary infections. Unlike other livestock production species, horses can live a long life and are expected to be reproductively sound in their later years. For these reasons, probably more so than other species, the safe amount of specific mycotoxins in feed is unknown for the horse. In addition to exposure, incidence of disease can also be influenced by the presence of multiple mycotoxins (Speijers and Speijers, 2004). Factors influencing susceptibility to mycotoxins include: disease, heat stress, marginal nutritional profile, drug interactions, presence of multiple toxins, crowding, age and reproductive status.

1.4. *Fusarium* Mycotoxins

1.4.1. Introduction

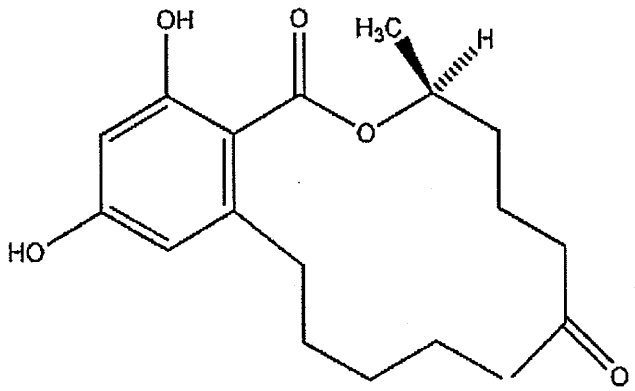
The genus *Fusarium* contains a large number of species of fungi, which produce various mycotoxins. There are more than 300 known *Fusarium* mycotoxins, but very little is known about the relative toxicity of most of these compounds. *Fusarium* mycotoxins of significant public health and agro-economic importance include trichothecenes, ZEN, fumonisins, moniliformin and fusaric acid (FA). The trichothecene mycotoxins are a group of over 180 compounds produced by *Fusarium, Stachybotrys, Myrothecium* and other fungal genera. The name trichothecene was derived from the fungus *Trichothecium* and these compounds are chemically related sesquiterpenoids that all possess a tetracyclic 12,13-epoxy-trichothec-9-ene ring system (Figure 1). Deoxynivalenol (DON) is considered the most common trichothecene but also one of the least toxic. Other prominent trichothecenes include T-2 toxin (considered one of the least prevalent but most toxic), diacetoxyscirpenol, Fusarenon-X and satratoxins. ZEN, fumonisins, moniliformin and FA are classified as non-trichothecene *Fusarium* mycotoxins (Figure 2). Most trichothecenes are found in very small quantities. The trichothecenes are known as feed refusal toxins due to loss of appetite being one of the first observable symptoms (Trenholm et al., 1994). These compounds have been shown to alter brain neurochemistry by increasing tryptophan and serotonin levels by inhibition of hepatic protein synthesis resulting in

Figure 1. Structure of trichothecene mycotoxins.

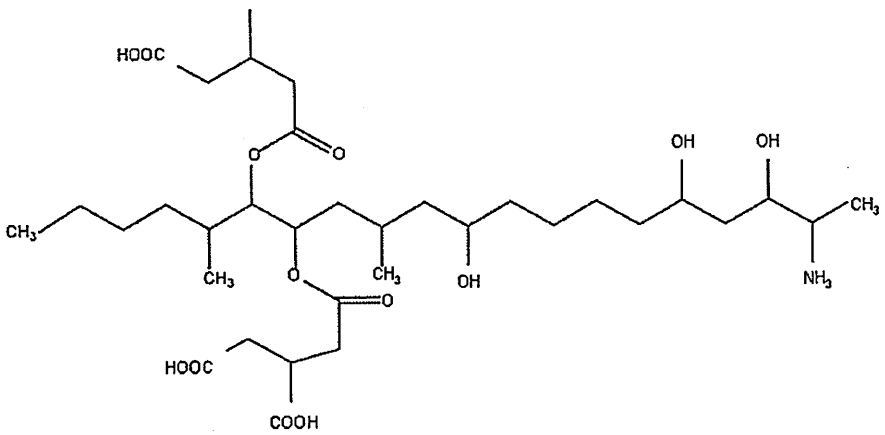


TOXIN	R1	R2	R3	R4	R5
T-2	$(CH_3)_2CHCH_2COO$		OAc	OAc	OH
HT2	$(CH_3)_2CHCH_2COO$		OAc	OH	OH
A-T2	$(CH_3)_2CHCH_2COO$		OAc	OAc	OAc
DON	=O	OH	OH		OH
NIV	=O	OH	OH	OH	OH
3ADON	=O	OH	OH		OAc

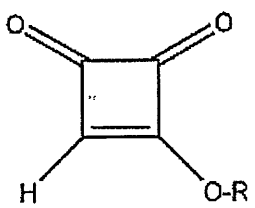
Figure 2. Structure of non-trichothecene mycotoxins.



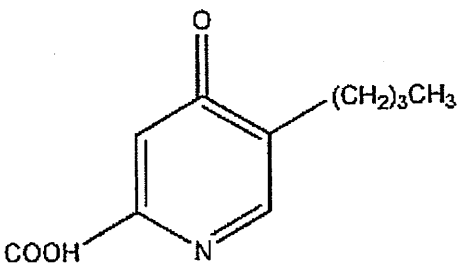
2a) ZEARALENONE



2b) FUMONISIN B1



2c) MONILIFORMIN



2d) FUSARIC ACID

hyperaminoacidemia (Meloche and Smith, 1995 and Wannemacher and Dinterman, 1983). Excess brain serotonin, a neurotransmitter synthesized from tryptophan, can contribute to loss of appetite, lethargy, sleepiness and loss of muscle co-ordination (Rossi-Fanelli and Cangiano, 1991). Studies in laboratory animals and pigs have shown enhanced serotonergic activity in the brain as one of the possible mechanisms for DON and fusaric acid induced feed refusal, vomiting and other behavioral changes (Smith et al., 1997).

1.4.2. Deoxynivalenol and Fusaric acid

Immunosuppression and the increased susceptibility to infectious diseases are the main features of chronic exposure of animals to low levels of trichothecene mycotoxins (Rotter et al., 1994). It has been shown that impairment of immunity against bacterial and parasitic infection, as well as susceptibility to disease, increases after mycotoxin consumption (Venturini et al., 1996). The immunosuppressant effects of ingested mycotoxins can also induce secondary diseases. In mice, ingestion of DON may also cause glomerulonephritis (kidney failure) from the overproduction of IgA immunoglobulins in the intestines, which accumulates in the kidneys. Johnson et al. (1997) fed barley containing 36-44 ppm DON (as-fed basis) to horses while at pasture. No detectable effects were found specifically on feed intake or serum IgA concentrations during the 40 days of the trial using non-pregnant mares and geldings. It was suggested that the horse may not be susceptible to the adverse effects of DON and the horse's gastric microflora may be able to detoxify it.

Toxicological synergism has also been reported among *Fusarium* mycotoxins (Smith et al., 1997). Studies have indicated that the presence of fusaric (5-butylpicolinic) acid, a compound synthesized from tryptophan by *Fusarium* molds, will increase the growth suppression seen when low levels of DON are fed to starter pigs (Smith et al., 1997). Although fusaric acid (FA)

was chemically characterized years ago, it has not been considered to be a significant factor in *Fusarium* mycotoxicoses because of its relatively low toxicity. Fusaric acid is pharmacologically active, however, and alters brain neurochemistry in a wide range of animal species. A physiological effect of fusaric acid is a decrease in blood pressure. Such an affect could be of significance for the horse as one of its production parameters is athletic performance. Like DON, fusaric acid is associated with increased brain serotonin levels but through a different mechanism. Trichothecenes elevate brain serotonin levels by elevating total blood tryptophan. Fusaric acid increases the availability of this tryptophan to the brain. Fusaric acid is a tryptophan analogue and competes with tryptophan for binding to blood albumin therefore an increase in free tryptophan in the blood occurs (Chaouloff et al., 1986). It has been demonstrated that fusaric acid and DON act synergistically to reduce feed consumption and trigger loss of muscle coordination and lethargy in starter pigs (Smith et al., 1997).

Horses may come in contact with DON and/or fusaric acid through the consumption of corn, barley, oats, wheat, wheat bran, soybean hulls or meal, wheat or distillery by-products in grain concentrates and hay. An often overlooked potential source of equine exposure to mycotoxin contamination is bedding, with straw being a common source. Contamination of bedding material represents a risk through ingestion, inhalation and dermal contact. The amount of DON in straw depends primarily on the presence of contaminated grain and chaff, two sources known to have more concentrated levels of mycotoxins. Horses fed a grain ration containing significant levels of DON may also consume their bedding. If the bedding contains a significant level of toxin, the total DON consumption could exceed a tolerance threshold. Additional exposure could come from dried or pasture forage. Conversely, the practice of the inclusion of a large forage component as compared to concentrates in the traditional equine diet may provide some

protection from mycotoxin exposure as a high concentration of plant fiber has been shown to reduce some of the deleterious effects of mycotoxins (Carson and Smith, 1983; James and Smith, 1982).

A published case report cited weight loss and elevated hepatic enzymes in horse serum with the probable cause being straw contaminated with DON (Zeyner et al., 2002). Approximately 50% of 104 Warmblood-type riding horses stabled in Germany experienced sudden weight loss. Further examination of nine of the affected horses revealed marked elevation of liver enzyme activity of both GDH and GGT. Analysis of feed, hay and bedding revealed DON concentrations ranging from 0.5 to 2.7 ppm in the straw. Once the horses were removed from the contaminated bedding they slowly gained weight and general condition progressively recovered (Zeyner et al., 2002). The FDA levels of concern for DON are 2ppm for wheat entering the milling process for humans and 1ppm for the finished product for humans. The level of concern for DON in wheat for livestock is 4ppm (Wood, 1992).

1.4.3. Zearalenone

Zearalenone (ZEN) is an estrogenic *Fusarium*, non-trichothecene mycotoxin. ZEN binds to estrogen binding sites and has been shown to cause enlargement of the uterus and rectal and vaginal prolapse, abortions and infertility with pre-pubertal animals appearing to be more sensitive than older animals and swine being the most susceptible livestock species (D'Mello, et al., 1999; Kalleta and Ettala, 1981). ZEN toxicity is more readily recognized than trichothecene toxicity because these clinical signs are very specific.

Juhasz et al. (2001) reported that ZEN was administered to mares for ten days at 7 mg of purified ZEN per os daily to mimic a 1 ppm concentration in the feed. This intake had no adverse effect on the reproductive parameters of cyclic mares but skin lesions were observed

around the mouth in 3 of 6 horses. Gimeno and Quintanilla (1983) reported a case of natural outbreak of ZEN mycotoxicosis on a commercial horse farm. Corn screenings fed to the horses produced strong estrogenic symptoms after a feeding period of 30 days. Clinical signs in the mares included: feed refusal, prolapsed uteruse and internal hemorrhage. Severe flaccidity of the genitals was observed in the males. It was found that the corn screenings fed to the horses contained on average 2.7 ppm zearalenone. No other common mycotoxins were found. Reports of feeding zearalenone-contaminated diets to pigs indicated that 1 ppm is the minimum concentration required to produce hyperestrogenism (James and Smith, 1982). Although cattle are traditionally considered to be more resistant to the effects of zearalenone than other species the ingestion of zearalenone contaminated hay has been associated with abortion in cows (Kalleta and Ettala, 1981).

1.4.4. Fumonisins

Fumonisins are produced primarily by *Fusarium moniliforme* and *F. proliferatum,* the *Fusarium* species that invade and are prevalent in corn. Fumonisins are recently discovered mycotoxins that can impair immune function, cause kidney and liver damage, decrease animal performance and cause death. In pigs, fumonisin has been linked with porcine pulmonary edema (PPE) (Harrison et al., 1990). While fumonisin ingestion in horses can cause equine leukoencephalomalacia (ELEM), which is typified by staggers, stupor, lameness, seizure (due to brain necrosis) and death. Fumonisin levels associated with PPE and ELEM ranging from 1 to 330 ppm fumonisin B_1 (FB_1) (PPE) and 1 to 126 ppm FB_1 (ELEM), respectively (Ross et al., 1991). Additional studies (Ross et al., 1992) suggested that fumonisin B_1 concentrations greater than 10 ppm in horse feeds made those animals consuming the feed likely candidates for ELEM.

Although the ingestion of naturally contaminated feed containing fumonisin has been shown to elevate brain serotonin levels, it appears that fumonisin did not initiate this effect.

Smith and MacDonald (1991) demonstrated fusaric acid would increase tryptophan, serotonin and 5-hydroxyindoleacetic acid in the brains of pigs. Previous work by Porter and coworkers (1990) examined corn associated with ELEM on tryptophan levels in rat tissues and found elevated concentrations, however, these investigators did not measure fusaric acid levels in the corn. Subsequent work by Porter did not show similar effects with the feeding of purified FB_1 (Porter, et al., 1993). It seems probable that fumonisin does not affect brain serotonin levels but toxins produced along with fumonisin seem to be responsible for this activity. Fumonisins interfere with sphingolipid metabolism, disrupting endothelial cell walls and basal membranes (van der Westhuizen et al., 2001). Sphingolipids are one of the primary groups of lipids in cell membranes. They have multiple functions, including signal transduction, cell growth, cell-to-cell communication, immunorecognition, as well as aiding in defining the physical nature of lipoproteins. There are three common fumonisin mycotoxins designated as FB_1, FB_2 and FB_3. FB_1 is well documented as the primary cause of leukoencephalomalacia in horses. Fumonisin B_1 has also been documented to alter cardiovascular and hepatic function and elevate serum cholesterol (Smith et al., 2002; Haschek et al., 2001). FB_2 has also been implicated as a possible cause of ELEM, however, the only trial examining this toxin (75 ppm FB_2) in the equine also had levels of FB_1 of 3 ppm (Ross, et al., 1994). Two of three ponies consuming this diet for 136 days demonstrated symptoms of ELEM. Ponies receiving a diet containing 75 ppm FB_3 and less than 1 ppm of both FB_1 and FB_2 for 56 days were clinically normal with no differences from control ponies at the time of necropsy. From this trial it is clear that FB_3 is not as toxic as FB_1

and FB_2. The effects of FB_2 are not as obvious due to the somewhat high concentrations of FB_1 in the diet containing 75 ppm FB_2.

In the horse, the consumption of fumonisins produces neurological symptoms associated with multi-focal liquefactive necrosis of the white matter affecting multiple horses in a herd. Once clinical signs appear, the majority of affected horses die and horses that survive typically have some degree of permanent neurological dysfunction. Corn screenings can be heavily contaminated with fumonisins and should never be fed to horses. Concentrations of FB_1, FB_2 and FB_3 in equine feed should not exceed 5 ppm and contaminated materials should not exceed 20% of the diet on a dry-matter basis

1.4.5. T-2 Toxin

Studies on the effects of T-2 toxin on the horse are sparse, with only one report involving the feeding of T-2 toxin. The feeding of 7 mg of purified T-2 toxin to horses per os daily, to mimic a 1 ppm concentration in feed, had no effect on the ovarian activity of mares (Juhasz et al., 1997). In poultry, swine and laboratory animals, oral and intestinal lesions are frequently observed in animals consuming T-2 or diacetoxyscirpenol (DAS) at levels as low as 100 ppb for a period of 25 days (Sklan et al, 2001).

1.5. Mycotoxins and Colic

Barnett et al. (1995) examined the relationship between mycotoxins and equine colic. Feed samples from farms experiencing possible feed-related colics (n=16) and control farms (n=10) were analysed. DON was found in the concentrate of 100% of colic cases (range 0.20 to 8.3 ppm) and 70% of the concentrate of control animals (n=10) (range 0-2.5 ppm). T-2 toxin at levels greater than 0.5 ppm and zearalenone greater than 0.7 ppm were present in 31% and 44% of the colic concentrate samples respectively, while neither were found in control samples.

Forage samples were not provided by all farms. Hence the causal-effect relationship of DON and colic in horses was not clear.

It is important to emphasize that in the above study commercial enzyme linked immunosorbent assays (ELISA) test kits were used for the analysis. These kits are not validated for the complex matrices found in forage samples. ELISA procedures are prone to false positive results on non-validated matrices and should be confirmed using HPLC and/or TLC testing.

1.6. Strategies to Prevent Mycotoxicoses

A practical approach to the prevention of mycotoxicoses in livestock is dietary inclusion of selective adsorbents that bind mycotoxins and thereby decrease their bioavailability (Huwig et al., 2001). The use of adsorbents such as activated charcoal, silicates, bentonites, clays and zeolites, in preventing mycotoxicosis has been extensively studied in livestock exposed to dietary mycotoxins (Ramos et al., 1996). These compounds have sometimes proven impractical due to high dietary inclusion rates. Glucomannan polymer derived from *Saccharomyces cerevisiae*[1026] is an organic adsorbent. Glucomannan polymer improved weight gain and feed intake and reduced organ weights in broiler chickens fed aflatoxins (Swamy and Devegowda, 1998) and aflatoxins and T-2 toxin (Raju and Devegowda, 2000). It has been shown that the supplementation of *Fusarium* mycotoxin-contaminated diets with glucomannan polymer prevented some of the mycotoxin-induced alterations in hematology, serum chemistry, biliary IgA concentrations and brain neurotransmitter concentrations in broiler chickens and pigs (Swamy et al., 2002a,b, 2003).

CHAPTER 2

GENERAL EXPERIMENTAL RATIONALE, HYPOTHESIS AND OBJECTIVES

2.1 Experimental Rationale

Deoxynivalenol, FA and ZEN are the most commonly occurring *Fusarium* mycotoxins in feedstuffs grown in Canada. Studies using both purified *Fusarium* mycotoxins and from naturally-contaminated grains have shown that livestock species are sensitive to *Fusarium* mycotoxicoses but limited work has been done with horses. The effects of chronic exposure of horses to low doses of *Fusarium* mycotoxins are unknown. A major concern, especially for elite athletes or breeding stock, is that long-term exposure to low levels of mycotoxins may affect performance or breeding ability without the appearance of overt clinical signs.

Historically, mycotoxins were identified by their acute affects in the horse. Chronic exposure to low doses of mycotoxins, however, is likely more common. The goal of the current project is to examine effects on equine health and performance, *in vivo*, which could be associated with the exposure to chronic doses of *Fusarium* mycotoxins.

2.2 Experimental Hypothesis

1. The feeding of grains naturally contaminated with *Fusarium* mycotoxins reduces feed intake and alters weight maintenance of horses.

2. The feeding of grains naturally contaminated with *Fusarium* mycotoxins alters the metabolism of horses.

3. The feeding of grains naturally contaminated with *Fusarium* mycotoxins alters athletic responses of horses.

4. Dietary supplementation with GM polymer is beneficial in preventing *Fusarium* mycotoxicoses in horses.

2.3 Objectives

1. To determine the responses of horses when fed diets containing a blend of grains naturally-contaminated with *Fusarium* mycotoxins specifically in terms of:

    (a) Feed intake and weight maintenance

    (b) Serum biochemistry, and haematology

    (c) Athletic response as determined using a time to fatigue treadmill step test.

2. To determine the efficacy of orally administered organic polymers in preventing *Fusarium* mycotoxicoses in horses.

CHAPTER 3

AN INVESTIGATION OF THE CONCENTRATIONS OF SELECTED FUSARIUM MYCOTOXINS AND THE DEGREE OF MOLD CONTAMINATION OF ONTARIO FIELD-DRIED HAY.

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3.1. Abstract

The levels of selected mycotoxins and mold contamination of Ontario field-dried hay from 10 performance horse farms were examined. The farm owner, trainer or manager was questioned regarding their opinion of the quality of the hay. Half of the hay sampled showed potentially significant levels of mycotoxins, mold and actinomycete contamination. Subjective opinion did not correlate with objective analysis. Deoxynivalenol (vomitoxin), T_2 toxin and zearalenone were measured with vomitoxin present in the highest amounts. Vomitoxin is among the mycotoxins most frequently found as contaminants in cereal crops, in temperate climates, in North America. It can be concluded that the levels found in this study could potentially have an influence on the health of horses consuming such hay.

3.2. Introduction

Forage is the basis of any feeding program for the horse. The type, quality and amount of forage determines what additional concentrates or supplements should be fed. The feeding of forage can also influence the behavior of the stabled horse (Houpt, 1990; Houpt and McDowell, 1993; Pond et al., 1995) Fibre is important for normal gut function and for the stabled horse to

help to decrease the incidence of stable vices such as wood chewing (McCall, 1993). Long stemmed hay is a traditional source of forage for horses, however its quality can vary. The nutritional status of hay can be altered through production and storage (Johnson et al., 1984).

Moldy forage can contribute to a range of disorders in the horse. Molds and actinomycetes can cause primary allergic and inflammatory respiratory disease, as well as influencing the incidence, severity and duration of episodes of infectious respiratory disease (Clarke, 1993). Inhaled respirable particles have been shown to compromise the ability of the respiratory system to clear inhaled contaminants, including bacteria, from the lung (Erlich 1980; Oberdorster 1995). Infectious respiratory disease can likewise lower the tolerance of the lung to inhaled contaminants (Willoughby et al., 1991). Mold can contribute to disorders by the production of mycotoxins. Mycotoxins may contribute to reproductive, immunological, respiratory, gastrointestinal and other disorders in livestock including the horse (Gimeno and Quintanilla, 1983; Hintz, 1991; Barnett et al., 1995, Casteel et al., 1995). Mycotoxins can also act as immunosuppressants thereby contributing to secondary mycotoxic diseases.

Molds are present in the field before harvest and can proliferate if moisture contents remain high through inadequate processing and storage conditions. Mold and subsequent mycotoxin contamination of a forage can increase in extreme environmental conditions such as droughts or rain, followed by cold weather, or from mechanical damage to the forage (Gregory et al., 1963; Smith and Seddon, 1998). The goal of the current study was to examine the levels of selected mycotoxins and mold contamination of Ontario field-dried hay being fed to performance horses after a storage period of approximately 11 months.

3.3. Materials and Methods

3.3.1. Forage Samples

Alfalfa-timothy mixed hay was sampled from ten Southwestern Ontario performance horse farms after a storage period of approximately 11 months during May of 1996. Samples of hay were taken from pooled core samples from 12 bales at each farm. All hay sampled was purchased or grown from different sources. Alfalfa and timothy were present in the bales of the samples in varying amounts. The farm owner, trainer or manager was asked a series of questions by the principal researcher from a questionnaire. Questions included, their opinion of the quality of the hay, if it was bought or made on farm, if it was field-dried and if it was routinely watered before feeding.

3.3.2. Mycology

The samples were washed with sterile water and aseptically direct plated as indicated below. Mesophilic fungi were counted and identified after 7 days incubation at 25C on malt extract glucose agar (MEA, Difco Laboratories, Detroit, M.I. USA) supplemented with 35 mg/l of streptomycin (Sigma, St. Louis, M.O. USA) to inhibit growth of bacteria and with 35 mg/l of rose Bengal (BDH Laboratory Supplies, Poole, England) to inhibit growth of fungi (Russel, 1974). Xerophilic fungi were counted and identified after 5 days incubation at 25C on dichloran glycerol agar (Oxoid, Hants, England). Thermotolerant fungi were counted and identified following 5 days at 40C on MEA supplemented with streptomycin and rose Bengal. Mesophilic bacteria were counted after 7 days on tryptone glucose yeast extract (Difco Laboratories, Detroit, M.I. USA) at 25C. Thermophilic actinomycetes were counted and identified after an incubation period of 3 days on half strength nutrient agar at 55C (Corbaz et al., 1963).

3.3.3. Mycotoxins

The concentrations of the mycotoxins; vomitoxin, T_2 toxin and zearalenone were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) (Veratox, Neogen Corporation, E.

Lansing, M.I, USA) in the laboratory of Central Lab Services of Ralston Purina Canada Inc. (Abouzied et al., 1991 and Bennett et al., 1994). Briefly, the samples were ground and mycotoxins extracted with distilled water. Each assay was performed with standards containing mycotoxins with known concentrations and a control. Mycotoxin concentrations were calculated by comparing the light absorbencies of the samples with those of known standards (6 % CV).

3.4. Results and Discussion

Nine of the ten farms bought their hay and one produced hay on the farm. Three of the farms considered the hay to be of good quality and seven felt that it was of poor quality. The hay was routinely soaked on two of the farms, one other if a horse had a respiratory problem and one other if it was considered particularly dusty.

The results of the microbial assessment of the ten hay samples are presented in Tables 3.1 and 3.2. Half of the hay samples showed potentially significant levels of mycotoxins, mold and actinomycete contamination. The concentrations of; vomitoxin, T2 toxin and zearalenone are presented in Table 3.3. Hay from farms 3, 5, 7 and 9 were found to have high mycotoxin levels and/or mold contamination. On visual examination it was found that these samples had high levels of alfalfa as compared to timothy suggesting that hay with a higher level of alfalfa had a higher chance of becoming moldy. Traditionally, alfalfa has been baled at a higher moisture content than timothy to prevent leaf shatter and moisture content is a key factor influencing mold growth.

Table 3.1. Bacteria and actinomycetes identified in hay samples collected from 10 different farms.

Farm	Mesophilic bacteria (cfu[A]/g)	Thermophilic actinomycetes (cfu/g)
1	1.6×10^5	none (<100)

2	5.4×10^5	none (<100)	
3	6.0×10^6	none (<100)	
4	7.1×10^5	none (<100)	
5	6.8×10^6	none (<100)	
6	2.5×10^6	none (<100)	
7	1.6×10^7	Total Count	820
		Thermoactinomyces candidus	820
8	1.3×10^6	Total Count	300
		Thermoactinomyces candidus	200
		Thermoactinomyces vulgaris	100
9	1.6×10^7	none (<100)	
10	4.3×10^4	Total Count	1200
		Thermoactinomyces candidus	1100
		Thermoactinomyces vulgaris	100

[A] cfu = colony forming units

Table 3.2. Mold identified in hay samples collected from 10 different farms.

Farm	Mesophilic fungi (cfu[A]/g)		Xerophilic fungi (cfu/g)		Thermophilic fungi (cfu/g)	
1	none	< 1000	none	< 1000	none	< 1000
2	none	< 1000	Total Count	7000	none	< 1000
			Scopulariopsis sp.	4000		
			non-sporulating fungi	3000		
3	Total Count	5000	Total Count	4000	none	< 1000
	Eurotium sp.	5000	*Aspergillus niger*	1000		
			Eurotium sp.	3000		
4	Total Count	2.2×10^4	Total Count	3.1×10^4	none	< 1000
	Alternaria sp.	2.2×10^4	*Alternaria sp.*	2.9×10^4		
			Fusarium sp.	2000		
5	Total Count	7.6×10^4	Total Count	1.1×10^6	none	< 1000
	Alternaria sp.	2.7×10^4	*Alternaria sp.*	1.0×10^5		
	Fusarium sp.	2.1×10^4	*Cladosporium sp.*	1.0×10^4		
	Sphaeropsidales sp.	2.7×10^4	*Fusarium sp.*	5.3×10^5		
	Rhodotorula sp.	1000	*Sphaeropsidales sp.*	3.9×10^5		
			Verticicladium sp.	4.0×10^4		
6	none	< 1000	Total Count	3600	none	< 1000
			Wallemia sp.	3600		
7	Total Count	1000	Total Count	1.2×10^5	none	< 1000
	Alternaria sp.	1000	*Alternaria sp.*	2700		
			Aureobasidium sp.	1000		
			Sphaeropsidales sp.	1.2×10^5		
8	none	< 1000	Total Count	1000	none	< 1000
			Aspergillus glaucus	1000		
9	Total Count	2.3×10^4	Total Count	3.5×10^6	none	< 1000
	Alternaria sp.	1.5×10^4	*Alternaria sp.*	7.0×10^4		
	Aspergillus glaucus	1000	*Fusarium sp.*	2.3×10^5		
	Yeasts	1000	*Cladosporium sp.*	1.0×10^4		
	non-sporulating fungi	6000	*Sphaeropsidales sp.*	3.2×10^6		
10	Total Count	2000	Total Count	1000	none	< 1000
	Alternaria sp.	1000	non-sporulating fungi	1000		
	Cladosporium sp.	1000				

[A] cfu = colony forming units

Table 3.3. Mycotoxin levels (ppm) found in hay samples collected from 10 different farms.

Farm	Vomitoxin	T_2 toxin	Zearalenone
1	1.20	< 0.15	< 0.25
2	1.30	0.30	< 0.25
3	2.60[B]	0.40	0.31
4	2.10	0.30	0.28
5	2.80[A]	0.30	1.21
6	1.90	0.30	0.33
7	3.60[A]	0.40	0.41
8	2.00	0.30	0.25
9	2.70[A]	0.40	< 0.25
10	1.20	0.40	0.31

[A] = evidence of heavy mold contamination based on the number of bacteria, actinomycetes and mold colony forming units

[B] = evidence of moderate mold contamination based on the number of bacteria, actinomycetes and mold colony forming units

Vomitoxin was present in the highest amounts of the three mycotoxins measured. *Fusarium sp.* is the main source of this mycotoxin but other molds including *Cephalosporium sp.*, *Myrothecium sp.*, *Stachybotrys sp.* and *Trichoderma sp.* can also produce it. *Fusarium sp.* was not one of the significant mold genera found except in sample 5. *Fusarium sp.* could have been present prior to storage and could have been destroyed through the succession of microorganisms during the storage period. Samples from farms 5, 7 and 9 were heavily contaminated with bacteria, actinomycetes and mold and the sample from farm 3 was moderately contaminated with storage fungi such as *Eurotium sp.*

Vomitoxin is a member of the trichothecene family of mycotoxins. Consumption of these compounds has been associated with loss of appetite, vomiting, lesions of the intestinal tract, immunosuppression, lethargy and ataxia in domestic animals and man (Smith and Seddon,

1998). Vomitoxin has also been shown to contribute to haemorrhagic disorders, cause digestive upsets including vomiting and diarrhea in swine and may contribute to reproductive, gastrointestinal, immunological, respiratory and various other disorders in livestock including the horse (Barnett et al., 1995 and Lacey, 1975). Vomitoxin is among the most frequent trichothecene contaminants found on cereal crops in the United States (Wood, 1992). FDA levels of concern for vomitoxin for wheat are 2 ppm for wheat entering the milling process for humans and 1 ppm for the finished product for humans. The level for wheat for livestock is 4 ppm (Wood, 1992). As a comparison, the levels found in this study could potentially have an influence on the health of horses consuming such hay. The threshold of significant biological activity is unknown for the horse, however chronic exposure to lower than acute levels may contribute to a wide range of disorders. Toxicological synergism has also been reported among Fusarium mycotoxins (Smith et al., 1997).

Half of the hay samples showed potentially significant levels of mycotoxins, mold and actinomycete contamination. Subjective opinion did not correlate with objective analysis, thus confirming that the level of mold contamination is not always easy to judge as was reported by Kotimaa et al. (1984). Molds and actinomycetes common in stable dust and used in inhalation challenge tests on COPD-horses have been confirmed to be causes of respiratory hypersensitivity (McGorum et al., 1993). Even minor degrees of airway inflammation, broncho-constriction or elevated mucous production will compromise the equine athlete (Clarke et al., 1987). Several management approaches, including the choice of forage, can have an affect on the air quality in the stable (Curtis et al., 1996 and Raymond et al., 1997). Recognition of the many associations between fungi and ill health is growing. Horner et al. (1995) have thoroughly outlined fungal

allergens and Flannigan and Miller (1995) stressed the influence of fungi in human health (Horner et al., 1995 and Flannigan and Miller, 1995).

The data acquired in this study, albeit preliminary, indicates the importance of further investigations into current and new production and storage methods of hay, especially alfalfa and alternative forage products. Further investigations are needed to establish the role that chronic exposure to mycotoxins has on the horse.

3.5. Conclusions

Half of the hay samples showed potentially significant levels of mycotoxin and mold contamination. Subjective opinion did not correlate to objective analysis, thus confirming that the level of mold contamination is not always easy to judge visually. Of the mycotoxins measured, vomitoxin was present in the highest amounts. Vomitoxin is among the most frequent mycotoxin contaminant found on cereal crops in the United States. FDA levels of concern for vomitoxin for wheat are 2 ppm for wheat entering the milling process for humans and 1 ppm for the finished product for humans. The level for wheat for livestock is 4 ppm. It can be concluded that the levels found in this study could potentially have an influence on the health of horses consuming such hay. Further investigations are needed to establish the role that exposure to mycotoxins has on the horse.

CHAPTER 4

EFFECTS OF FEEDING A BLEND OF GRAINS NATURALLY CONTAMINATED WITH FUSARIUM MYCOTOXINS ON FEED INTAKE, SERUM CHEMISTRY, AND HEMATOLOGY OF HORSES, AND THE EFFICACY OF A POLYMERIC GLUCOMANNAN MYCOTOXIN ADSORBENT.

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4.1. Abstract

The feeding of *Fusarium* mycotoxin-contaminated grains adversely affects the performance of swine and poultry. Very little information is available, however, on adverse effects associated with feeding these mycotoxin-contaminated grains on the performance of horses. An experiment was conducted to investigate the effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, serum immunoglobulin (Ig) concentrations, serum chemistry and hematology of horses. A polymeric glucomannan mycotoxin adsorbent (GM polymer) was also tested for efficacy in preventing *Fusarium* mycotoxicoses. Nine mature, non-exercising, light, mixed breed mares were assigned randomly to one of three dietary treatments for 21 d. The horses were randomly re-assigned and the experiment was subsequently replicated in time following a 14 d washout interval. Feed consumed each day was a combination of up to 2.8 kg of concentrates and 5 kg of mixed timothy/alfalfa hay. The concentrates fed included: (1) control, (2) blend of contaminated grains (36% contaminated wheat and 53% contaminated corn)

and (3) blend of contaminated grains + 0.2% GM polymer. Diets containing contaminated grains averaged 15.0 ppm deoxynivalenol, 0.8 ppm 15-acetyldeoxynivalenol, 9.7 ppm fusaric acid and 2.0 ppm zearalenone. Feed intake of all horses fed contaminated grains was reduced ($P < 0.001$) compared to controls throughout the experiment. Supplementation of 0.2% GM polymer to the contaminated diet improved ($P = 0.004$) feed intake of horses compared to those fed the unsupplemented contaminated diet. Serum activities of gamma-glutamyltransferase were higher ($P = 0.047$ and 0.027) in horses fed the diet containing contaminated grain compared to those fed the control diet on d 7 and 14, but not on d 21 ($P = 0.273$). Supplementation of GM polymer to the contaminated diet reduced ($P < 0.05$) serum gamma-glutamyltransferase activities of horses compared to those fed unsupplemented contaminated diet on d 7 and 14. Other hematology and serum chemistry measurements including serum IgM, IgG and IgA were not affected by diet. It was concluded that the feeding of grains naturally contaminated with *Fusarium* mycotoxins caused a reduction in feed intake and altered serum gamma glutamyltransferase activities. The supplementation of GM polymer prevented these mycotoxin-induced adverse effects.

4.2. Introduction

Fusarium fungi are commonly found in temperate climates and *Fusarium* mycotoxins are likely the most economically significant grain mycotoxins on a global basis (Wood, 1992). *Fusarium* mycotoxins, however, have also been found to contaminate pastures and forages. Among the trichothecene mycotoxins, deoxynivalenol (DON, vomitoxin) is a major contributor to reduced feed intake in animals. Trichothecene mycotoxins have also been shown to be potent immunosuppressive agents (Bondy and Pestka, 2000).

Pigs are the most sensitive species to feeding DON and it has been shown that feeding DON-contaminated grains reduces feed intake and weight gain of pigs (Trenholm et al., 1994). A

synergistic interaction between DON and fusaric acid (FA) on growth rate of pigs fed blends of naturally contaminated grains has been demonstrated (Smith et al., 1997). Very little information is available, however, on the toxicity associated with the feeding of *Fusarium* mycotoxin-contaminated grains on feed intake and metabolism of horses. Johnson et al, (1997) found no effect on feed intake, serum chemistry or hematology when horses were fed barley naturally contaminated with 36 to 44 ppm of DON for 40 d.

Polymeric mycotoxin adsorbents have been reported to prevent the deleterious effects of mycotoxins by reducing intestinal absorption of mycotoxins and preventing subsequent transport to target tissues (Ramos et al., 1996). A polymeric glucomannan mycotoxin adsorbent (GM polymer) has shown beneficial effects in preventing *Fusarium* mycotoxicoses in poultry and swine (Swamy et al., 2002a, b; Raju and Devegowda, 2000).

The objectives of the current experiment were to investigate the effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on feed intake, serum immunoglobulin (Ig) concentrations, serum chemistry and hematology of horses. A polymeric mycotoxin adsorbent (GM polymer) was also tested for efficacy in preventing *Fusarium* mycotoxicoses.

4.3. Materials and Methods

4.3.1. Experimental Animals and Diets

Mature, non-exercising, light, mixed breed mares (n = 9) were assigned randomly to one of three dietary treatments for 21 d. The horses were randomly re-assigned and the experiment was subsequently replicated in time following a 14 d washout interval. Feed consumed each day was a combination of up to 2.8 kg of concentrates and 5 kg of mixed timothy/alfalfa hay (Table 4.1) formulated to meet the nutritional requirements of a mature, non-working horse (NRC, 1989). The concentrates fed included: (1) control, (2) blend of contaminated grains (36% contaminated

wheat and 53% contaminated corn) and (3) blend of contaminated grains + 0.2% GM polymer (MTB-100, Alltech Inc., Nicholasville, KY). Water was provided ad libitum.

Table 4.1. Composition of dietary concentrates and hay (as-fed basis)

Item	Control[a]	Mycotoxin[b]	0.2% GM[c]	Hay
Ingredient, %				
Uncontaminated corn	53.60	-	-	-
Uncontaminated wheat	35.80	-	-	-
Contaminated corn	-	53.60	53.40	-
Contaminated wheat	-	35.80	35.80	-
Soybean meal (47.5% CP)	8.80	8.80	8.80	-
Vitamin and mineral mixture[d]	1.80	1.80	1.80	-
Glucomannan polymer	0.00	0.00	0.20	-
Nutrient composition				
Crude protein[e], %	13.21	14.44	14.30	15.82
DE, Mcal/kg[f]	2.30	2.30	2.30	1.73
Ca,%[f]	0.29	0.29	0.29	0.38
P,%[f]	0.24	0.24	0.24	0.18

[a]Diet with control corn and wheat.

[b,c]Mycotoxin-contaminated diets supplemented with 0 and 0.2% glucomannan polymer, respectively.

[d]Provided : calcium, 3.3%; phosphorus, 2.0%; sodium 4.0%; magnesium, 0.5%; potassium, 1.3%; iodine, 10mg/kg; copper, 520mg/kg; sulphur, 0.2%; iron, 1866mg/kg; zinc, 1570mg/kg; cobalt, 6mg/kg; fluorine, 100mg/kg; vitamin A (retinyl palmitate), 114400IU/kg; vitamin K_3, 47mg/kg; thiamin, 87mg/kg; riboflavin, 84mg/kg; pantothenic acid, 258mg/kg; niacin, 410mg/kg; pyridoxine, 65mg/kg; choline, 1690mg/kg; folic acid, 19mg/kg; biotin, 3545ug/kg; vitamin B_{12}, 348ug/kg.

[e]Analyzed (AOAC, 1980).

[f]Calculated.

The horses were housed at the Equine Research Centre, Guelph, ON, in individual 3.6 x 3.6 m boxstalls with 8 hr of group turnout on paddocks with minimal pasture a day. Horses were examined daily for any adverse clinical signs.

4.3.2. Animal Care

This experiment was reviewed and approved by the University of Guelph Animal Care Committee. Animals were managed and cared for according to the guidelines of the Canadian Council on Animal Care.

4.3.3. Analysis of Dietary Mycotoxins

Dietary and bedding materials were analyzed for DON, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, T-2 toxin, iso T-2 toxin, acetyl-T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenon-X, diacetoxyscirpenol, scirpentriol, 15-acetoxyscirpentriol, neosolaniol, zearalenone, zearalenol and fumonisin using a combination of gas chromatography and mass spectrometry (North Dakota State University, Fargo, ND). The detection limit for these mycotoxins was 0.2 ug/g. Briefly, the mycotoxins were extracted from 25 g of ground sample

with 100 ml acetonitrile:water (84:16) for 1 h on a horizontal shaker. A 6 ml aliquot of the supernatant was gravity filtered through 1.5 g of C_{18}:alumina (1:1) and a 2 ml aliquot of the eluent was evaporated at 65°C for 30 min. The residue was derivatized (*N*-trimethylsilyimidazole (TMSI) + trimethylchlorosilane (TMCS) + *n,o*-bis[trimethylsily]triflouroacetamide (BSTFA) + pyridine) to form trimethylsily (TMS) ester derivatives of trichothecenes and estrogens. The TMS-mycotoxin derivatives were separated on a gas chromatograph (Shimadzu, QP 5050), using a Restek (Restek Corp, Bellefonte, PA) 30 m RTX 35 x 0.25 mm x 0.25 μm phase capillary column, and assayed by select ion monitoring (SIM) and electron ionization (EI) in a mass spectrometer, using 3 or 4 ion fragments for identification and quantification of each mycotoxin. The capillary column was held at 90°C for 1 min after on column injection, heated to 210°C at 40°C/min, and then heated to 310°C at 5°C/min and held for 1 min (25 min total) before recycling. Helium linear velocity at 110°C was 40 cm/s.

Dietary and bedding material aflatoxin contents (G_1, G_2, B_1 and B_2) were analyzed by high performance liquid chromatography (HPLC; Animal Health Laboratory, Laboratory Services Division, University of Guelph, Guelph, ON). The detection limit for these mycotoxins was 0.002 ug/g (Tarter et al., 1984). Fusaric acid content was determined by HPLC method of Matsui and Watanabe (1988) as modified by Smith and Sousadias (1993) and confirmed by Porter et al. (1995; 0.77) ug/g detection limit.

4.3.4. Experimental Parameters Studied

4.3.4.1 Feed Intake and Weight Gain. At each feeding (0800 and 1600 hour) the quantity of concentrate consumed by each horse was recorded. The horses were weighed weekly during the trial.

4.3.4.2. Hematology and Serum Biochemical Analysis. Blood samples were drawn by jugular venipuncture (7, 14 and 21 d). Blood samples were subjected to hematology and serum chemistry determinations (Laboratory Services Division, University of Guelph, Guelph, ON).

Red blood cell (RBC) count, mean corpuscular volume (MCV) and hematocrit were determined and mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentrations (MCHC) were calculated. Hemoglobin was measured as cyanomethemoglobin after lysing the red blood cells using an Advia 120 Hematology System (Bayer Inc., Healthcare Division, Toronto, ON). Complete blood cell counts (differential leukocyte count) were performed manually to test for changes in absolute numbers of leukocytes (WBC), lymphocytes, segmented neutrophils, banded neutrophils, monocytes, eosinophils and basophils.

Serum concentrations of total protein, albumin, globulin, glucose, beta-hydroxybutyrate, haptoglobin, urea, cholesterol, creatinine, bilirubin, calcium, phosphorus, magnesium, sodium, potassium, chloride, and activities of alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and creatine kinase (CK) were determined using a Hitachi 911 autoanalyzer (Roche Diagnostics, Hoffman-La Roche Ltd, Montreal, QC).

4.3.4.3. Serum immunoglobulin concentrations. Concentrations of IgA, IgM and IgG were determined in serum samples obtained on day 21 using the radial immunodiffusion technique (RID) of Mancini et al., 1965 (Animal Health Lab Services, University of Guelph, Guelph, ON). Briefly, serum was placed into plates containing buffered agarose with monospecific antisera. The plates were incubated at room temperature for 18 to 24 h. The diameter of the subsequent diffusion ring was used to determine the concentration by comparing to a standard curve.

4.3.5. Statistical Analysis

Data were subjected to Levene's homogeneity of variances test before the analysis for treatment differences. Data were analyzed by ANOVA using the GLM procedure of SAS as a completely randomized design within the period (SAS Inst. Inc., Cary, NC). Effects of period and period and diet interaction were tested in the model, and the same were removed from the model when found non-significant. The effect of feeding *Fusarium* mycotoxin-contaminated diets was determined by employing a simple contrast between the horses fed the control diet and those fed the mycotoxin-contaminated diet. The ability of the GM polymer to prevent *Fusarium* mycotoxin-induced effects was tested by simple contrasts between the horses fed the mycotoxin-contaminated diet with and without 0.2% GM polymer and, between horses fed the control diet and the GM polymer supplemented diet (Kuehl, 2000). Statements of statistical significance were based on $P < 0.05$.

4.4. Results

4.4.1. Dietary mycotoxin concentrations

The analyzed concentrations of mycotoxin in the diets are given in Table 4.2. DON and FA were found in the control diet while only DON was detected in the straw bedding. Zearalenone and 15-acetyl DON were detected in the mycotoxin-contaminated diets with and without GM polymer in addition to DON and FA.

Table 4.2. Mycotoxin content of experimental diets, hay and bedding material

Dietary Group	Mycotoxins[a]			
	DON[b]	FA[c]	ZEA[d]	15-ADON[e]
	ug/g	ug/g	ug/g	ug/g
Control[f]	0.7	5.4	<0.1	<0.2
Mycotoxin[g]	14.1	6.4	2.0	0.7
0.2% GM[h]	15.9	12.9	2.0	0.8
Hay	<0.1	12.3	<0.1	<0.2
Straw Bedding	1.2	3.9	<0.1	<0.2

[a]Other mycotoxins: i.e. T-2 toxin, iso T-2 toxin, acetyl T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenon-X, 3-acetyl deoxynivalenol, nivalenol, diacetoxyscirpenol, scirpentriol, 15-acetyl scirpenol, neosolaniol, zearalenol, fumonisin B_1, and aflatoxins (G_1, G_2, B_1 and B_2) were below detection limits (2 ppm for fumonisin B_1, 0.01 ppm for aflatoxins and 0.2 ppm for the other mycotoxins).

[b]Deoxynivalenol.

[c]Fusaric acid.

[d]Zearalenone.

[e]15-acetyldeoxynivalenol

[f]Diet with control corn and wheat.

[g,h]Mycotoxin-contaminated diets supplemented with 0 and 0.2% glucomannan polymer, respectively.

4.4.2. Feed Intake and Changes in Body Weight

Feeding of the contaminated diet to horses resulted in reduced feed intake compared to those fed the control diet throughout the experiment (Table 4.3; $P < 0.001$). Supplementation of 0.2% GM polymer to the contaminated diet improved ($P = 0.004$) feed intake of horses compared to those fed the unsupplemented contaminated diet but not as compared to control diet (Table 4.3). Consumption of forage remained unaffected regardless of diet fed (data not shown). Body weights of horses were unaffected by diet (Table 4.4).

Table 4.3. Concentrate intake of horses fed blends of grains naturally contaminated with *Fusarium* mycotoxins[a]

	Average daily intake (kg)				
Dietary group	0 - 7d	0-14d	0-21d	7-14d	14-21d
Control[b]	2.80	2.80	2.80	2.80	2.80
Mycotoxin[c]	1.03	1.02	1.00	1.01	0.96
0.2% GM[d]	1.81	1.71	1.64	1.60	1.52
SEM	0.09	0.08	0.07	0.08	0.06
Control vs Mycotoxin[e]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Mycotoxin vs 0.2% GM[f]	0.004	0.011	0.009	0.032	0.011
Control vs 0.2% GM[h]	0.001	0.001	< 0.001	0.001	< 0.001

[a]All dietary treatments included 5kg of mixed timothy/alfalfa hay per day.

[b]Values are least-square means; n = 6 horses.

[c]Concentrate with control corn and wheat.

[d]Mycotoxin-contaminated concentrate containing 36% contaminated wheat and 53% contaminated corn.

[e]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

[f,g,h]Simple contrasts comparing Control diet with Mycotoxin diet, Mycotoxin diet with 0.2% GM, and Control diet with 0.2% GM respectively.

Table 4.4. Mean body weights of horses throughout the experiment

Diet	Initial weight, kg	d 7	d 14	d 21
Control[a]	457	459	457	460
Mycotoxin[b]	480	477	473	475
0.2% GM[c]	482	480	480	479
SEM	15	15	15	14

[a]Diet with control corn and wheat.

[b]Mycotoxin-contaminated concentrate containing 36% contaminated wheat and 53% contaminated corn.

[c]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

4.4.3. Hematology and serum chemistry

Serum activities of gamma-glutamyltransferase were higher (P = 0.047 and 0.027) in horses fed the contaminated diet compared to those fed the control diet on d 7 and 14, but not on d 21 (P = 0.273; Table 4.5). Supplementation of the contaminated diet with 0.2% GM polymer significantly decreased (P = 0.008 and 0.005) the increase in serum activities of gamma-glutamyltransferase on d 7 and 14, but the decrease was not significantly different from the control diet. Other hematology and serum chemistry parameters were not significantly affected by the dietary treatments (Table 4.6, 4.7 and 4.8).

Table 4.5. Effect of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on serum gamma-glutamyltransferase activity levels (U/L) in horses

Diet	GGT[a]		
	7 d	14 d	21 d
Control[b]	11.44[h]	12.23	11.49
Mycotoxin[c]	22.98	23.86	15.27
0.2% GM[d]	8.57	9.58	8.24
SEM	1.88	1.69	1.35
Control vs Mycotoxin[e]	0.047	0.027	NS[i]
Mycotoxin vs 0.2% GM[f]	0.008	0.004	NS[i]
Control vs	NS[i]	NS[i]	NS[i]

0.2% GM[g]

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[a]Serum glutamyltransferase activity (U/L).

[b]Diet with control corn and wheat.

[c]Mycotoxin-contaminated concentrate containing 36% contaminated wheat and 53% contaminated corn.

[d]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

[e,f,g]Simple contrasts comparing Control diet with Mycotoxin diet, and Mycotoxin diet with 0.2% GM, respectively.

[h]Values are least-square means; n = 6 horses.

[i]Not significant.

Table 4.6. Effect of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on selected serum protein fractions in horses

Diet	TP[a]			Alb[b]			Glob[c]		
	7 d	14 d	21 d	7 d	14 d	21 d	7 d	14 d	21 d
Control[d]	68.05[h]	70.57	68.38	31.79	32.85	31.96	36.27	37.72	36.41
Mycotoxin[e]	70.63	72.63	71.12	31.32	32.33	31.98	39.31	40.29	39.13
0.2% GM[f]	68.98	70.95	70.31	31.73	32.64	32.54	37.26	38.30	37.77
SEM[g]	1.01	0.98	0.99	0.29	0.35	0.36	1.15	1.12	1.09

[a]Total proteins, g/L.

[b]Albumin, g/L.

[c]Globulin, g/L.

[d]Concentrate with control corn and wheat.

[e]Mycotoxin-contaminated concentrate containing 36% contaminated wheat and 53% contaminated corn.

[f]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

[g]No significant effect of diet on serum protein concentrations was observed ($P > 0.05$).

[h]Values are least-square means; n = 6 horses.

Table 4.7. Effect of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on d 21 serum immunoglobulin concentrations (g/L) in horses

Diet	IgA[a]	IgM[b]	IgG[c]	Total Ig[d]
Control[e]	2.98[i]	0.65	20.19	23.82
Mycotoxin[f]	3.14	0.64	25.40	29.18
0.2% GM[g]	3.41	0.74	28.39	32.54
SEM[h]	0.66	0.06	2.06	2.43

[a,b,c,d] Serum Immunoglobulin A, M, G and total, respectively.

[e]Diet with control corn and wheat.

[f]Mycotoxin-contaminated diet containing 36% contaminated wheat and 53% contaminated corn.

[g]Mycotoxin-contaminated diet supplemented with 0.2% glucomannan polymer.

[h]No significant effect of diet on serum immunoglobulin concentrations was observed ($P > 0.05$).

[i]Values are least-square means; n = 6 horses.

Table 4.8. Effect of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on d 21 complete and differential white blood cell counts in horses

Diet	WBC[a]	SNC[b]	LC[c]	MC[d]	EC[e]
Control[f]	7.05[j]	4.07	2.46	0.27	0.13
Mycotoxin[g]	7.20	3.46	3.26	0.26	0.17
0.2% GM[h]	6.83	3.32	3.01	0.25	0.19
SEM[i]	0.20	0.20	0.21	0.02	0.02

[a]White blood cell count, 10^9/L.

[b]Segmented neutrophil count, 10^9/L.

[c]Lymphocyte count, 10^9/L.

[d]Monocyte count, 10^9/L.

[e]Eosinophil count, 10^9/L.

[f]Concentrate with control corn and wheat.

[g]Mycotoxin-contaminated concentrate containing 36% contaminated wheat and 53% contaminated corn.

[h]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

[i]No significant effect of diet on differential leukocyte count was observed ($P > 0.05$).

[j]Values are least-square means; n = 6 horses.

4.4.4. Serum immunoglobulin concentrations

Serum IgG, IgA and IgM concentrations were not affected by diets (Table 4.6).

4.5. Discussion

4.5.1. Dietary mycotoxin concentrations

The variation in the level of DON (14.1 and 15.9 ppm) in the two contaminated diets may have been due to uneven distribution of mycotoxins in the grain and the limitations of mixing (Davis et al., 1980). Infestation of grains by *Fusarium* molds is dependent on appropriate microbial conditions for growth and can occur sporadically. It was reported that the same level of inclusion of contaminated grains resulted in 1.9 ppm DON in one experiment and 4.4 ppm DON in another (Smith et al., 1997). A deoxynivalenol concentration of 0.7 ppm in the control diet and 1.2 ppm in the straw bedding in the current experiment provides an illustration of the mycotoxin contamination of Ontario-grown feedstuffs. Contamination of bedding material represents a risk to the stabled horse both through ingestion and inhalation. Respiratory disorders associated with exposure to bedding material contaminated with mold and trichothecene mycotoxins have been reported in agricultural workers and livestock, including horses (Andrassy et al., 1979; Forgacs, 1972). Exposure to bedding contaminated by molds has long been associated with lower airway inflammation and subsequently poor performance of the athletic horse (Eyre, 1972; Thomson and McPherson, 1984).

The variation in the FA concentrations in the contaminated diets and presence in the control diet may be caused, in part, from contaminated soybean meal (Smith and Sousadias, 1993; Smith et al., 1997). Matsui and Watanabe (1988) reported that soybean plants can be contaminated with FA. Bacon et al., (1996) stressed that given the numerous common *Fusarium* species that produce FA, the natural occurrence of this compound as a contaminant in foods and feeds should be considered commonplace.

The presence of FA in the test diets, whether contributed from contaminated grains or contaminated soybean meal, needs to be addressed in the context of possible synergistic interactions between FA and DON. Smith et al., (1997) reported a synergistic interaction between DON and FA on weight gains of pigs. Fusaric acid has a very low acute toxicity compared to trichothecene mycotoxins (Hidaka et al., 1969). Fusaric acid (Smith and MacDonald, 1991) and DON (Prelusky, 1993) have been shown to elevate pig brain concentrations of serotonin, albeit through different mechanisms, which can lead to loss of appetite, lethargy, and muscle co-ordination (Leathwood, 1987).

Reports of feeding zearalenone-contaminated diets to pigs indicated that 1 ppm is the minimum concentration required to produce hyperestrogenism (James and Smith, 1982). The minimum concentration required to produce symptoms in the horse is unknown. A field outbreak of zearalenone mycotoxicosis in horses was associated with corn screenings containing approximately 2.6 ppm of zearalonene (Gimeno and Quintanilla, 1983). The content of deoxynivalenol or FA was not determined. The dietary zearalenone content of about 0.7 ppm in the current experiment should not have caused metabolic effects in the horses tested. *Fusarium graminearum* fungi can produce deoxynivalenol and zearalenone simultaneously in infected corn and wheat (Cote et al., 1985). Toxicological synergism between DON and zearalenone has not been observed in swine (Cote et al., 1985) or mice (Forsell et al., 1986).

4.5.2. Feed Intake and Changes in Body Weight

It was reported that feeding barley naturally contaminated with DON (36 – 44 ppm) to horses did not cause feed refusal (Johnson et al., 1997). These results are in contrast to those of the current experiment in which a reduction in feed intake was observed in horses fed contaminated grain containing 14 ppm DON. Johnson et al. (1997) fed only one source of contaminated grain

as compared to the current study in which a blend of naturally contaminated grains was used. A blend would more likely contain combinations of mycotoxins that could act synergistically to decrease feed intake. It was reported that the horses were fed 2.7 kg of contaminated barley per day, an amount of grain similar to that consumed in the current study, but it is unclear if the contaminated barley was mixed with other uncontaminated feed ingredients. The researchers did not report levels of FA or other possible *Fusarium* mycotoxins, and did not provide information on the use of a control diet or nutritional information on the diet used.

Results of the current experiment suggest a relatively high degree of reduced feed intake when horses are fed concentrates containing a blend of grains naturally contaminated with *Fusarium* mycotoxins. Trichothecene mycotoxins inhibit cellular protein synthesis to varying degrees. This property is likely the cause of many of the pathologies associated with trichothecene toxicosis. T-2 toxicosis results in hyperaminoacidemia (Wannemacher and Dinterman, 1983) and this is likely due to inhibition of hepatic protein synthesis (Meloche and Smith, 1995). Subsequent elevations in blood tryptophan can result in increased concentrations of tryptophan in the brain. Tryptophan is the precursor of the neurotransmitter serotonin and the serotonergic neurons are thought to be important mediators of behaviors such as appetite, muscle coordination and sleep. Serotonin synthesis in the brain is poorly regulated and can be promoted by increased intracellular concentrations of tryptophan (Leathwood, 1987).

Body weights of horses in the current experiment were unaffected by diet. The horses used were mature and non-exercising. It is likely that each supplementation period of 21 d was not long enough for weight loss on the maintenance diet fed.

4.5.3. Hematology and serum chemistry

Little work has been reported regarding the effect of feeding *Fusarium* mycotoxin-contaminated grains to horses on hematologic or serum biochemical parameters. Serum activities of the hepatic membrane associated enzyme, gamma-glutamyltransferase were higher in horses fed the contaminated diet when compared to control diet on d 7 and 14, but not on d 21. These findings are in contrast to those of Johnson et al. (1997). Such differences may be due to the previously described differences in experimental protocols. An increase in the activity of this enzyme indicates either hepatocellular damage or enzyme induction. The lack of differences found in serum activities of gamma-glutamyltransferase on d 21 implies that the horses may have adapted to the hepatotoxicity caused by the combination of *Fusarium* mycotoxins.

4.5.4. Serum immunoglobulin concentrations

Limited information has been published on the possible immunomodulatory effects of feeding *Fusarium* mycotoxin-contaminated grains to domestic animals. Johnson et al. (1997) fed 36-44 ppm DON to horses and found no significant effects of diet on serum IgA and IgG concentrations. Serum IgM concentrations were not measured. These results are in agreement with the current experiment. Increased serum IgA and IgM concentrations were found in pigs fed the same blend of contaminated grains as the current study (Swamy et al., 2002b). Ingested DON affects intestinal immunoglobulin synthesis. Specifically, DON stimulates intestinal IgA production in mice, leading to an elevated concentration of circulating serum IgA (Dong et al., 1991 and Pestka et al., 1989).

4.5.5. Effect of GM polymer supplementation

The use of adsorbents such as activated charcoal, silicates, bentonites, clays and zeolites, in preventing mycotoxicosis has been extensively studied in livestock exposed to dietary

mycotoxins (Ramos et al., 1996). These compounds have sometimes proven impractical due, in part, to high dietary inclusion rates. Glucomannan polymer derived from *Saccharomyces cerevisiae*[1026] is an organic adsorbent. Glucomannan polymer improved weight gain and feed intake and reduced organ weights in broiler chickens fed aflatoxins (Swamy and Devegowda, 1998) and aflatoxins and T-2 toxin (Raju and Devegowda, 2000). Previous studies in our laboratory indicated that the supplementation of *Fusarium* mycotoxin-contaminated diets with GM polymer prevented some of the mycotoxin-induced alterations in hematology, serum chemistry, biliary IgA concentrations and brain neurotransmitter concentrations (Swamy et al., 2002a, b). The reduction in consumption of naturally contaminated grains was partially prevented by the feeding of GM polymer in the current study. The increase in serum gamma-glutamyltransferase activities when contaminated grain was fed was prevented with the inclusion of GM polymer suggesting that toxin absorption was reduced below the threshold of biological activity. Target organs associated with the mixture of mycotoxins present in the experimental diets can be liver, brain, and reproductive organs.

4.6. Implications

Horses chronically fed *Fusarium* mycotoxin-contaminated grains exhibited a reduction in feed consumption. This effect can have wide implications for the horse industry. Inclusion of contaminated grains in rations for horses should, therefore, be minimized.

Further research is required to determine the effect of *Fusarium*-induced feed reduction and metabolic changes on athletic performance of horses. The capability of glucomannan polymer to decrease the *Fusarium* induced reduction in feed intake and prevent metabolic changes shows promise for the horse industry.

CHAPTER 5

EFFECTS OF FEEDING A BLEND OF GRAINS NATURALLY-CONTAMINTED WITH FUSARIUM MYCOTOXINS ON FEED INTAKE, METABOLISM AND INDICES OF ATHLETIC PERFORMANCE OF EXERCISED HORSES.

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5.1. Abstract

An experiment was conducted to determine the effect of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins to mature, exercised horses, and to test the efficacy of a polymeric glucomannan mycotoxin adsorbent (GM polymer) in preventing *Fusarium* mycotoxicoses. Six mature, mixed breed mares with an average weight of 530 kg were assigned to one of three dietary treatments for 21 d in a replicated3 x 3 Latin square design. Feed consumed each day was a combination of up to 3.5 kg of concentrates and 5.0 kg of mixed timothy/alfalfa hay. The concentrates fed included: (1) control (2) blend of contaminated grains; and (3) contaminated grains + 0.2% GM polymer (MTB-100, Alltech Inc., Nicholasville, KY). Concentrates containing contaminated grains averaged 11.0 ppm deoxynivalenol, 0.7 ppm 15-acetyldeoxynivalenol, and 0.8 ppm zearalenone. Feed intake and body weight were monitored over a 21-d period. Horses were maintained on a fixed exercise schedule throughout the experiment. At the end of the experiment, each horse completed a time to fatigue treadmill step test. Variables measured were (1) time to fatigue (2) heart rate (3) hematological variables; and (4) serum lactate concentration during pre-test, and each step of the test, and 5 and 10 min post-

test were:. Each step consisted of 2 min of fast trot with a 2% increase in incline after each 2 min interval. Feed intake of horses fed contaminated grains was reduced compared to controls throughout the experiment ($P < 0.05$). Supplementation of 0.2% GM polymer to the contaminated diet did not alter feed intake of horses compared with those fed the unsupplemented contaminated diet ($P > 0.05$). All hay was consumed regardless of concentrate fed. Weight loss from 0 to 21 d was observed in horses fed contaminated grains as compared to controls ($P < 0.05$). No effect of diet was seen on variables used to measure athletic ability although the results showed an expected response to exercise for a fit horse. We concluded that exercised horses are susceptible to *Fusarium* mycotoxicoses as indicated by appetite suppression and weight loss.

5.2. Introduction

Mycotoxins are harmful secondary metabolites of fungi that can reduce performance of livestock (Smith, 1991). The occurrence of mold and mycotoxins in food and animal feed is a major problem globally. *Fusarium* fungi are commonly found in temperate climates and *Fusarium* mycotoxins are likely the most prevalent on a global basis (Wood, 1992).

The feeding of blends of grains naturally contaminated with *Fusarium* mycotoxins has been shown to reduce livestock and poultry performance including reduced feed intake and weight gain, and adverse metabolic, hematologic, and neurochemical changes (Swamy et al., 2002; 2004a,b). Very little information is available, however, on the toxicity associated with the feeding of *Fusarium* mycotoxin-contaminated grains to horses. Johnson et al. (1997) found no effect on feed intake, serum chemistry, or hematology when horses were fed barley naturally contaminated with 36 to 44 ppm deoxynivalenol (**DON**). This is in contrast to more recent findings which described reduced feed intake in unexercised horses fed a combination of

Fusarium mycotoxins (Raymond et al., 2003). Athletic ability is a major performance variable for the horse. Regular heavy exercise places metabolic stresses on the animal. A major concern, especially for elite athletes, is that the chronic feeding of low levels of mycotoxins may affect performance without the appearance of overt clinical symptoms.

Objectives of this study were to determine the effects of feeding grains naturally-contaminated with *Fusarium* mycotoxins on feed intake, weight maintenance, serum chemistry, hematology, and athletic performance of exercised horses, and the efficacy of an organic polymeric mycotoxin adsorbent in the prevention of *Fusarium* mycotoxicoses. The GM polymer has been shown to prevent many adverse effects of grain naturally contaminated with *Fusarium* mycotoxins in horses (Raymond et al., 2003), pigs (Swamy et al., 2002a,b), boilers (Swamy et al., 2004a) and layers (Chowdhury and Smith, 2004).

5.3. Materials and Methods

5.3.1. Experimental Animals and Diets

Mature, mixed breed mares with an average weight of 530 kg (n = 6) were randomly assigned to one of three dietary treatments for 21 d following a replicated 3 x 3 Latin square design with a 14 d treatment recovery interval during which time the control diet was fed. Feed consumed each day was a combination of up to 3.5 kg of concentrates and 5.0 kg of 50:50 timothy/alfalfa hay formulated to meet the nutritional requirements of a mature, medium-working horse (NRC, 1989) (Table 5.1). The concentrates fed included: (1) control, (2) blend of contaminated grains; and (3) blend of contaminated grains + 0.2% GM polymer (MTB-100, Alltech Inc., Nicholasville, KY). Water was provided ad libitum. The blend of contaminated grains consisted of 53.6% contaminated corn and 35.8% contaminated wheat.

Table 5.1. Composition of diets (as-fed basis)

Item	Control[a] concentrate	Mycotoxin[b] concentrate	0.2% GM[c] concentrate	Hay
Ingredient, %				
Uncontaminated corn	53.60	-	-	-
Uncontaminated wheat	35.80	-	-	-
Contaminated corn	-	53.60	53.40	-
Contaminated wheat	-	35.80	35.80	-
Soybean meal (47.5% CP)	8.80	8.80	8.80	-
Vitamin and mineral premix[d]	1.80	1.80	1.80	-
Glucomannan polymer[e]	0.00	0.00	0.20	-
Nutrient composition				
Crude protein[f], %	12.62	13.73	12.72	11.09

[a]Diet with control corn and wheat.

[b,c] Mycotoxin-contaminated diets supplemented with 0 and 0.2% glucomannan polymer, respectively.

[d]Supplied per kilogram of diet: calcium, 33 mg; phosphorus, 20 mg; sodium 40 mg; magnesium, 50 mg; potassium, 13 mg; iodine, 10 mg/kg; copper, 520 mg/kg; sulfur, 2 mg; iron, 1866 mg/kg; zinc, 1570 mg/kg; cobalt, 6 mg/kg; fluorine, 100 mg/kg; vitamin A (retinyl palmitate), 114400 IU/kg; vitamin K_3, 47 mg/kg; thiamin, 87 mg/kg; riboflavin, 84 mg/kg; pantothenic acid, 258

mg/kg; niacin, 410 mg/kg; pyridoxine, 65 mg/kg; choline, 1690 mg/kg; folic acid, 19 mg/kg; biotin, 3545 ug/kg; vitamin B_{12}, 348 ug/kg.

[e] MTB-100 (Alltech Inc., Nicholasville, KY).

[f] Analyzed (AOAC, 1980).

The horses were housed at the University of Guelph (Guelph, ON, Canada) in individual 3.6 m^2 boxstalls with 8 h of group turnout on paddocks with minimal pasture a day. The paddocks contained minimal grass growth due to both overgrazing and time of year that the study was conducted. Horses were examined daily for any adverse clinical signs.

The horses were fed concentrates twice a day (0800 and 1600). Any uneaten portion from the 0800 feeding was added to the 1600 feeding. One hour was allotted to the 0800 feeding before uneaten portion was removed. The concentrates fed at 1600 remained in the stall until 0800 and were then removed and weighed. The quantity of concentrate consumed by each horse was recorded once per day. The horses were weighed weekly during the trial (1500 h, 7, 14 and 21 d).

Horses were initially conditioned for a 3-wk period before the first supplementation phase. Horses were maintained on a fixed exercise schedule during the supplementation phase. This consisted of three 45 min workouts including 25 min of fast trot (18kph) a wk using an exerciser (Odyssey Performance Trainer, System Fencing, Guelph, ON). Each horse was contained in an individual gated area. The horse was encouraged to remain at the required pace using the gate located at the rear of the animal. At the end of the supplementation phase, each horse completed a time to fatigue treadmill step test. Horses were maintained on a reduced exercise schedule using an exerciser during each treatment recovery period. The reduced exercise schedule

consisted of one 30 minute workout of walking during the first wk followed by one 30 minute workout including 10 min of fast trot during the second wk.

5.3.2. Animal Care

The experiment was reviewed and approved by the University of Guelph Animal Care Committee. Animals were managed and cared for according to the guidelines of the Canadian Council on Animal Care.

5.3.3. Analysis of Dietary Mycotoxins

Diets and hay were analyzed for DON, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, T-2 toxin, iso T-2 toxin, acetyl-T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, fusarenon-X, diacetoxyscirpenol, scirpentriol, 15-acetoxyscirpentriol, neosolaniol, zearalenone, zearalenol, and fumonisin using a combination of gas chromatography and mass spectrometry (Shimadzu, QP 5050) (North Dakota State University, Fargo, ND) as described by Raymond et al. (2003). The limit of detection for fumonison $\mathbf{B_1}$ was 2.0 ppm and was 0.2 ppm for all other mycotoxins. Fusaric acid (**FA**) content (0.77 ppm limit of detection) was determined by the method of Matsui and Watanabe (1988) as modified by Smith and Sousadias (1993) and confirmed by Porter et al. (1995).

5.3.4. Experimental Parameters Studied

5.3.4.1. Hematology and Serum Biochemical Analysis.

Blood samples were drawn by jugular venipuncture (1500h, 7, 14 and 21 d).

Samples were drawn into a 7 ml sterile vacutainer containing EDTA using an 18 gauge needle. Red blood cell count (**RBC**, 0.0 to 7.0 10^9/L limit of detection), mean corpuscular volume (**MCV**, 76 to 100 fL expected value), and hematocrit (33 to 57% expected value) were

determined, and mean corpuscular hemoglobin (**MCH,** 24 to 31 pg expected value), and mean corpuscular hemoglobin concentrations (**MCHC,** 28 to 34 gL expected value) were calculated. Hemoglobin (0.0 to 225 g/L limit of detection) was measured as cyanomethemoglobin after lysing the red blood cells using an Advia 120 Hematology System (Bayer Inc., Healthcare Division, Toronto, ON). Complete blood cell counts (differential leukocyte count) with limits of detection were performed manually to test for changes in absolute numbers of leukocytes (**WBC,** 0.02 to 400 10^9/L), lymphocytes (16 to 44%), segmented neutrophils (40 to 77%), banded neutrophils (40 to 77%), monocytes (4 to 9%), eosinophils (1 to 7%), and basophils (0 to 1%).

Blood samples were drawn into a 7 ml sterile silicone-coated vacutainer. Serum concentrations and limits of detection of total protein (0 to 150 g/L), albumin (0 to 80 g/L), globulin (0 to 80 g/L), glucose (0 to 25 mmol/L), beta-hydroxybutyrate (0 to 3200 umol/L), haptoglobin (0 to 100 g/L), urea (0 to 50 umol/L), cholesterol (0 to 20 mmol/L), creatinine (0 to 1770 umol/L), bilirubin (0 to 513 umol/L), calcium (0 to 5 mmol/L), phosphorus (0 to 6.46 mmol/L), magnesium (0 to 2 mmol/L), sodium (0 to 300 mmol/L), potassium (0 to 200 mmol/L), chloride (0 to 300 mmol/L), and activities of alkaline phosphatase (**AP,** 0 to 500 U/L), glutamate dehydrogenase (**GLDH,** 0 to 80 U/L), aspartate aminotransferase (**AST,** 0 to 800 U/L), gamma glutamyltransferase (**GGT,** 0 to 1200 U/L), and creatine kinase (**CK,** 0 to 2300 U/L) were determined using a Hitachi 911 autoanalyzer (Roche Diagnostics, Hoffman-La Roche Ltd, Montreal, QC).

5.3.4.2. Time to Fatigue Treadmill Step Test.

At the end of the supplementation phase, each horse completed a time to fatigue step test on a high-speed treadmill. The step test consisted of a 5 min warm up at a walk, followed by 2 min of

slow trot (approximately 7 kph), 2 min of fast trot (approximately 15kph) then consecutive steps. Each step consisted of 2 min of fast trot with a 2% increase in incline after each 2 min. Time to fatigue was determined by the handler as the time when the horse was unwilling to maintain position on the treadmill for the second time. The test was terminated at this time and concluded with 10 min of cool down at a walk. Variables measured during pre-test, each step of the test, and 5 and 10 min post-test were: time to fatigue, heart rate, hematology, and serum lactate levels. Heart rate was recorded with a digital monitor (Polar Heart Rate Monitor, Equine Performance Group, Guelph, ON). A 14 gauge, 15 cm catheter was inserted into the left jugular vein before the time to fatigue treadmill step test. Once the catheter was inserted, it was secured with sutures and an extension set was attached to facilitate blood sampling during exercise. The catheter was kept patent with heparinized saline solution (10 U/mL). The catheter remained in place for approximately 2 h.

5.3.3. Statistical Analysis.

Experimental animals were assigned to different treatments in a Latin Square design. Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model included diet, horse, and period. The effect of feeding *Fusarium* mycotoxin-contaminated diets was determined by using a simple contrast between the horses fed the control diet and those fed the mycotoxin-contaminated diet. The ability of the GM polymer to prevent *Fusarium* mycotoxin-induced effects was tested by a simple contrast between the horses fed the mycotoxin-contaminated diet with and without 0.2% GM polymer. Differences were considered significant at $P < 0.05$. Feed intake over a period of wk for each treatment was added and statistical analyses were made on this total amount per wk. For cumulative feed intake, feed intake over the period of 21 d was considered.

5.4. Results

5.4.1. Dietary mycotoxin concentrations

The analyzed concentrations of mycotoxin in the concentrates and hay are given in Table 5.2. Deoxynivalenol was found in all concentrates whereas zearalenone and 15-acetyl DON were also detected in the concentrate containing contaminated grains. Only FA was detected in the hay.

Table 5.2. Mycotoxin content of concentrates and hay

Dietary Group	Mycotoxins[a]			
	DON[b]	FA[c]	ZEA[d]	15-ADON[e]
	ppm	ppm	ppm	ppm
Control[f]	0.4	<0.8	<0.1	<0.2
Mycotoxin[g]	11.2	<0.8	0.8	0.7
0.2% GM[h]	14.5	<0.8	0.9	0.7
Hay	<0.1	40.5	<0.1	<0.2

[a]Other mycotoxins were below detection limits.

[b]Deoxynivalenol.

[c]Fusaric acid.

[d]Zearalenone.

[e]15-acetyldeoxynivalenol

[f]Diet with control corn and wheat.

[g,h]Mycotoxin-contaminated diets supplemented with 0 and 0.2% glucomannan polymer, MTB-100 (Alltech Inc., Nicholasville, KY), respectively.

5.4.2. Feed Intake and changes in Body Weight

Feed intake was reduced in horses fed contaminated grain with or without 0.2% GM polymer compared to those fed the control diet throughout the study ($P < 0.05$) (Table 5.3). All hay offered was consumed regardless of diet fed (data not shown). One horse was removed from the trial after one treatment period during the 14-d recovery period due to colic symptoms determined to be unrelated to the trial.

Table 5.3. Feed intake of horses fed blended grain contaminated with Fusarium mycotoxins on feed intake (as-fed basis) (kg/horse[a])

Dietary group	0 to 7d	7 to 14d	14 to 21d	0 to 21d
Control[b]	24.5	24.5	24.5	73.5
Mycotoxin[c]	16.6	16.3	15.1	48.0
0.2% GM[d]	17.3	16.9	17.1	51.3
SEM	0.99	0.58	0.54	1.51
control vs mycotoxin[e]	0.015	0.001	0.001	0.001
mycotoxin vs 0.2% GM[f]	0.267	0.778	0.281	0.228

[a]Values are least-square means; n = 5 horses for mycotoxin and 0.2% GM diets. n = 6 for control diet.

[b]Concentrate with control corn and wheat.

[c]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat, and 53.6% contaminated corn.

[d]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer, MTB-100 (Alltech Inc., Nicholasville, KY).

[e,f]Simple contrasts comparing Control diet with Mycotoxin diet, and Mycotoxin diet with 0.2% GM respectively. Feed intake over a period of wk for each treatment was added and statistical analyses were made on this total amount per wk. For cumulative feed intake, feed intake over the period of 21 d was considered.

Weight loss over 0 to 21 d was observed in horses fed contaminated grains as compared to controls ($P < 0.05$) (Table 5.4). All animals lost weight over 0 to 7 d and 0 to 14 d while gaining weight over 7 to 14 d.

Table 5.4. Changes in body weight of horses fed blended grain contaminated with Fusarium mycotoxins[a]

		Weight change (kg/horse)				
Dietary group	Initial weight (kg)	0 to 7d	7 to 14d	14 to21d	0 to 14d	0 to 21d

Control[b]	534	- 2.6	3.0	- 3.6	0.4	- 3.2
Mycotoxin[c]	532	- 1.8	2.8	- 10.8	1.0	- 9.8
0.2% GM[d]	530	- 7.0	3.0	- 8.0	- 4.0	- 12.0
SEM		1.31	1.20	1.52	1.60	1.03
control vs mycotoxin[e]		0.704	0.972	0.170	0.774	0.036
mycotoxin vs 0.2% GM[f]		0.236	0.356	0.726	0.747	0.985

[a]Values are least-square means; n = 5 horses for mycotoxin and 0.2% GM diets. n = 6 for control diet.

[b]Concentrate with control corn and wheat.

[c]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[d]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer, MTB-100 (Alltech Inc., Nicholasville, KY).

[e,f]Simple contrasts comparing Control diet with Mycotoxin diet, and Mycotoxin diet with 0.2% GM respectively.

5.4.3. Hematology and serum chemistry

Hematology and serum chemistry variables (7, 14, and 21 d) were not significantly affected by the dietary treatments (Appendix I, II, and III).

5.4.4. Time to Fatigue Treadmill Step Test

No variables were affected by diet. The mean resting heart rates for control, mycotoxin, and 0.2 % GM horses were 40 bpm, 39 bpm, and 35 bpm, respectively (SEM = 1.39). The starting heart rates for control, mycotoxin, and 0.2 % GM horses were 125 bpm, 135 bpm, and 140 bpm, respectively (SEM = 4.33). The time of fatigue heart rates for control, mycotoxin, and 0.2 % GM horses were 173 bpm, 175 bpm, and 155 bpm, respectively (SEM = 4.80). The 5 min recovery heart rate for control, mycotoxin, and 0.2 % GM horses were 87 bpm, 82 bpm, and 79 bpm respectively (SEM = 2.68). The 10 min recovery heart rates for control, mycotoxin, and 0.2 % GM horses were 71 bpm, 75 bpm, and 72 bpm, respectively (SEM = 1.55). The times to fatigue for control, mycotoxin, and 0.2 % GM horses were 12.32 min, 11.14 min, and 9.94 min, respectively (SEM = 0.64) (Appendix IV).

The following blood variables were measured during the time to fatigue treadmill step test. The WBC measured at rest for control, mycotoxin, and 0.2 % GM horses were 8.32 10^9/L, 8.06 10^9/L, and 8.83 10^9/L, respectively (SEM = 0.34). The WBC measured at time of fatigue for control, mycotoxin, and 0.2 % GM horses were 11.56 10^9/L, 11.56 10^9/L, and 11.88 10^9/L, respectively (SEM = 0.38). The WBC measured at 10 min recovery for control, mycotoxin, and 0.2 % GM horses were 10.40 10^9/L, 10.56 10^9/L, and 11.18 10^9/L, respectively (SEM = 0.18). The LC measured at rest for control, mycotoxin, and 0.2 % GM horses were 3.66 10^9/L, 2.88 10^9/L, and 2.70 10^9/L, respectively (SEM = 0.20). The LC measured at time of fatigue for control, mycotoxin, and 0.2 % GM horses were 4.77 10^9/L, 4.42 10^9/L, and 4.31 10^9/L, respectively (SEM = 0.33). The LC measured at 10 min recovery for control, mycotoxin, and 0.2 % GM horses were 4.76 10^9/L, 4.02 10^9/L, and 4.13 10^9/L, respectively (SEM = 0.17). The RBC measured at rest for control, mycotoxin, and 0.2 % GM horses were 8.40 10^9/L, 7.84 10^9/L,

and 8.08 10^9/L, respectively (SEM = 0.11). The RBC measured at time of fatigue for control, mycotoxin, and 0.2 % GM horses were 11.41 10^9/L, 11.02 10^9/L, and 10.77 10^9/L, respectively (SEM = 0.08). The RBC measured at 10 min recovery for control, mycotoxin, and 0.2 % GM horses were 9.66 10^9/L, 9.08 10^9/L, and 9.13 10^9/L, respectively (SEM = 0.09). The HB measured at rest for control, mycotoxin, and 0.2 % GM horses were 142.20 g/L, 133.60 g/L, and 138.17 g/L, respectively (SEM = 1.90). The HB measured at time of fatigue for control, mycotoxin, and 0.2 % GM horses were 195.63 g/L, 188.40 g/L, and 185.50 g/L, respectively (SEM = 1.64). The HB measured at 10 min recovery for control, mycotoxin, and 0.2 % GM horses were 164.40 g/L, 156.20 g/L, and 158.00 g/L, respectively (SEM = 1.49). The HCT measured at rest for control, mycotoxin, and 0.2 % GM horses were 0.37 L/L, 0.35 L/L, and 0.36 L/L, respectively (SEM = 0.01). The HCT measured at time of fatigue for control, mycotoxin, and 0.2 % GM horses were 0.52 L/L, 0.49 L/L, and 0.48 L/L, respectively (SEM = 0.01). The HCT measured at 10 min recovery for control, mycotoxin, and 0.2 % GM horses were 0.43 L/L, 0.40 L/L, and 0.40 L/L, respectively (SEM = 0.00) (Appendix V).

The mean resting lactate levels for control, mycotoxin, and 0.2 % GM horses were 1.16 mmol/L, 1.18 mmol/L, and 1.15 mmol/L, respectively (SEM = 0.05). The time of fatigue lactate levels for control, mycotoxin, and 0.2 % GM horses were 3.78 mmol/L, 3.74 mmol/L, and 3.15 mmol/L, respectively (SEM = 0.08). The 5 min recovery lactate levels for control, mycotoxin, and 0.2 % GM horses were 2.32 mmol/L, 2.10 mmol/L, and 1.97 mmol/L, respectively (SEM = 0.08). The 10 min recovery lactate levels for control, mycotoxin, and 0.2 % GM horses were 2.04 mmol/L, 1.64 mmol/L, and 1.63 mmol/L, respectively (SEM = 0.05). The peak lactate levels for control, mycotoxin, and 0.2 % GM horses were 3.78 mmol/L, 3.74 mmol/L, and 3.25

mmol/L, respectively (SEM = 0.08). The times to peak lactate for control, mycotoxin, and 0.2 % GM horses were 12.35 min, 11.70 min, and 9.72 min, respectively (SEM = 0.66) (Appendix VI).

5.6. Discussion

5.6.1. Dietary mycotoxin concentrations

Failure to achieve similar concentrations of DON (11.2 and 14.5 ppm) in the two contaminated diets may have been due to uneven distribution of mycotoxins in the grain and the limitations of mixing (Davis et al., 1980). Infestation of grains by *Fusarium* molds is dependent on appropriate microbial conditions for growth and can occur sporadically.

The presence of FA in the hay needs to be addressed in the context of possible synergistic interaction between FA and DON. Smith et al., (1997) reported a synergistic interaction between DON and FA on weight gains of pigs. FA has a very low acute toxicity compared to trichothecene mycotoxins (Hidaka et al., 1969). FA (Smith and MacDonald, 1991) and DON (Prelusky, 1993) have been shown to elevate pig brain concentrations of serotonin, albeit through different mechanisms, which can lead to lethargy and loss of appetite, and muscle in-coordination. The absence of FA from the control and contaminated diets but presence in the hay is an illustration of the variations in amounts of metabolites produced by different *Fusarium* strains (Bacon et al., 1996).

The dietary zearalenone content in the current experiment should not have caused metabolic effects in the horses tested. The levels found in the current study are similar to those found previously (Raymond et al., 2003). The minimum concentration of zearalenone in contaminated diets required to produce hyperestrogenism in swine according to literature reports is 1 ppm (James and Smith, 1982). A field outbreak of zearalenone toxicosis in horses was associated

with the consumption of corn screenings containing approximately 2.6 ppm of zearalonene (Gimeno et al., 1983). The content of deoxynivalenol or FA was not reported. *Fusarium graminearum* fungi can produce deoxynivalenol and zearalenone simultaneously in infected corn and wheat (Cote et al., 1985). Toxicological synergism between DON and zearalenone has not been observed in swine (Cote et al., 1985) or mice (Forsell et al., 1986).

5.6.2. Feed Intake and Changes in Body Weight

Results of the current trial suggest a relatively high degree of reduced feed intake when horses are fed concentrates containing a blend of grains naturally contaminated with high levels of *Fusarium* mycotoxins. These results are in agreement with previous work (Raymond et al., 2003) but are in contrast to those reported by Johnson (1997) who found no effect on feed intake when feeding barley naturally contaminated with 36 to 44 ppm DON. This may be attributable to the synergistic effect of feeding a combination of mycotoxins found in blends of contaminated grains as has been observed for FA and DON in starter pigs (Smith et al, 1997). Johnson et al. (1997) fed only one source of contaminated grain as compared to the current study in which a blend of naturally contaminated grains was used. Horses exhibited a lower degree of reduced feed intake (35%) of contaminated grains during the current trial as compared to previous work (65%) (Raymond et al., 2003). This may be attributable to the increase in energy requirements due to exercise. The inability to maintain body weights in horses fed contaminated grains is in contrast to previous work (Raymond et al., 2003) despite consumption of greater amounts of contaminated grains. Weight loss exhibited in exercising horses when fed contaminated diets may affect athletic ability, but this was not evaluated at the beginning of this study and this conclusion cannot be made. Horses in the previous study were not maintained on an exercise schedule.

5.6.3. Time to Fatigue Treadmill Step Test.

No effect of diet was seen on time to fatigue. If the trial was longer or the exercise test more extreme, an effect might have been seen. As expected with exercise, there was an increase in serum lactate and heart rate followed by an appropriate decrease after recovery. This is in agreement with the findings of Sobotta et al. (2001) and Lindner at al. (2001). In the current study, heart rate was reaching 180 bpm at time of fatigue indicating the approach of the threshold from aerobic to anerobic metabolism. Serum lactate concentrations increased during exercise and decreased during recovery indicating the rapid removal of lactate by the recovering muscles and restoration of plasma volume. The results showed an expected response to exercise for a fit horse.

5.6.4. Hematology and serum chemistry

Serum activities of the hepatic membrane associated enzyme, gamma-glutamyltransferase, were not significantly different in horses fed the contaminated diet when compared to control diets. These findings are in contrast to earlier findings where higher levels were found in horses fed contaminated grains when compared to controls (Raymond et al., 2003). Such differences may be due to an increase in liver function found in exercising horses when compared to non-exercising animals as exhibited by increased clearance rates of xenobiotics, specifically antipyrine as a means to measure changes in hepatic drug metabolism (Dyke et al., 1998). The feeding of similarly contaminated grains to pigs resulted in a reduction in the weights of both liver and kidney (Swamy et al., 2002a,b).

5.6.5. Effect of GM polymer supplementation

The use of adsorbents such as activated charcoal, silicates, bentonites, clays, and zeolites, in preventing mycotoxicosis has been extensively studied in livestock exposed to dietary

mycotoxins (Ramos et al., 1996). The feeding of GM polymer in previous studies resulted in increased consumption of *Fusarium* contaminated equine concentrates (Raymond et al., 2003). This effect was not seen in the current study. The increase in dietary energy requirements due to the inclusion of the exercise regime resulted in a lesser degree of feed refusal than was seen previously. This reduced the potential for the GM polymer to significantly increase concentrate intake.

CHAPTER 6

GENERAL DISCUSSION

Moldy feeds and forage can contribute to a range of disorders in the horse. Molds and actinomycetes can cause primary allergic and inflammatory respiratory disease, as well as influencing the incidence, severity and duration of episodes of infectious respiratory disease. Inhaled respirable particles have been shown to compromise the ability to clear inhaled contaminants, including bacteria, from the lung. Likewise infectious respiratory disease can lower the lung's tolerance to inhaled contaminants. Until recently, studies investigating the effects of mycotoxins on horses were limited to trials with few animals or depended on extrapolations using data from other species (the pig or ruminant being the most common choices).

A series of experiments were conducted to compare the effects of feeding diets naturally-contaminated with *Fusarium* mycotoxins on both exercising and non-exercising mature horses. The contaminated diets were formulated by replacing corn and wheat of the control diet with those naturally-contaminated with *Fusarium* mycotoxins. The parameters chosen for the comparison were feed intake, and weight maintenance, serum chemistry, and haematology and athletic response as determined using a time to fatigue treadmill step test.

In the current study mature non-exercising horses were fed a diet in which the contaminated concentrates averaged 15 ppm DON. The contaminated concentrates also contained on average 0.8 ppm 15-acetyldeoxynivalenol, 9.7 ppm FA and 0.2 ppm ZEN. The control horses were consuming 2.8 kg concentrates and 5 kg of mixed hay per day. The contaminated grains used in this study were a mixture of naturally contaminated wheat and corn. Naturally contaminated

mixed sources are very important as opposed to pure forms or single contaminated sources. The former mimics more closely what is likely to be found in industry where an animal is not likely to be exposed to only one mycotoxin. The grains used most commonly in horse feeds are corn, oats and barley. In this study consumption of the contaminated diet reduced feed intake. It was also found that serum activities of gamma-glutamyltransferase (GGT) were significantly higher in horses consuming contaminated grain sampled on day 7 and 14 of supplementation but not for day 21. An increase in GGT levels indicates an effect on liver function without obvious signs of damage since GGT is a hepatic membrane-associated enzyme. In the current study involving an exercise program results were comparable with the addition of significant weight loss observed over the 21 day supplementation period in horses fed contaminated grains as compared to control animals.

Contamination of bedding material represents a risk through ingestion, inhalation and dermal contact. An unplanned DON concentration of 1.2 ppm in the straw bedding in the current feeding trial is an illustration of the potential mycotoxin contamination.

Forage is the basis of most feeding programs for the horse, with long-stemmed, field dried hay being the traditional source. Any horseperson can recognize the dust aerosol opening a bale of hay that has a high mold count and many individuals equate a high mold count with mycotoxin content. In the current study, subjective opinion of horse owners about their hay did not correlate with objective analyses of mycotoxins, mold and actinomycete contamination. It is important to note, as shown in the current studies, that a forage or feed with a low level of measurable mold contamination may still contain significant amounts of mycotoxins. Mycotoxin production does not correlate with mold growth and conditions that support the synthesis of one mycotoxin may actually inhibit the synthesis of another. Increased mycotoxin contamination and

the potential for mixtures of mycotoxins to be co-contaminants in feeds are now even more likely due to improved global grain transportation systems and global trading of agricultural commodities.

Mycotoxins are best known for their ability to cause acute, severe disease in horses. However, the impact of mycotoxins is much wider. Subclinical problems such as loss of performance and decreased reproductive capacity are non-specific disorders that can be linked with mycotoxin ingestion. Mycotoxins may be unavoidable contaminants in feeds and forages due to the unpredictability of pre-harvest contamination of susceptible crops by fungi. Various genera of fungi can produce mycotoxins whenever optimal conditions of temperature, humidity and suitable substrate prevail in a given area. It can be argued that given the complex interactions of mycotoxins with feed ingredients, varying exposure situations and the state of the individual horse, safe levels cannot be identified and that no levels of mycotoxins can be demonstrated to be safe in a field situation. Levels can be reduced with the testing of feeds and forages, increased awareness, proper management and possible the use of compounds that have been shown to have binding capabilities on mycotoxins.

6.1. Conclusions

Horses fed *Fusarium* mycotoxin-contaminated grains exhibit a reduction in feed consumption. The degree of the response is dependant on energy requirements dictated by exercise regimes. This is reflected by a lesser degree of reduced feed intake. Inclusion of *Fusarium* mycotoxin-contaminated grains in rations for horses should be minimized. Since these papers represent a large range in potential toxic levels, it is suggested that, until more precise research is completed, the maximum tolerable level for DON in the total feed for horses be comparable to levels allowed for humans. The maximum acceptable concentration of DON in

wheat intended for human consumption in flour is 1 ppm. The capability of glucomannan polymer to decrease *Fusarium*-induced reduction in feed intake and prevent metabolic changes shows promise but additional work is needed. Considerable research has been carried out on acute and chronic toxicity of some pure mycotoxins in a range of laboratory and domestic animal species but sub-clinical toxicological effects, such as, immunosuppression, and possible synergistic influences need attention. Additional work is needed to investigate chronic exposure of naturally contaminated grains in trials that extend past 21 days and in which further investigate athletic response. Additional work is also needed to investigate the effect of field levels of selected mycotoxins on systemic and immune function in the horse.

REFERENCES

Abouzied, M.M., M.N. Beremand, S.P. McCormick, J.J. and Pestka. 1991. Reactivity of deoxynivalenol (vomitoxin) monoclonal antibody towards putative trichothecane precursors and shunt metabolites. J. Food Protection 54: 288-290.

Andrassy, K., I. Horvath, T. Lakos, and Z. Toke. 1979. Mass incidence of mycotoxicosis in Hajdu-Bihar county. Mykosen 23:130-133.

AOAC. 1980. Official methods of analysis. 13th ed. Assoc. Offic. Anal. Chem., Washington, DC.

Bacon, C. W., J. K. Porter, W. P. Norred, and J. F. Leslie.. 1996. Production of fusaric acid by *Fusarium* species . Appl. Environ. Microbiol. 62:4039-4043.

Barnett, D.T., R.A. Mowery, W.M. Hagler, D.G. Bristol, and R.A. Mansmann. 1995. The correlation of selected mycotoxins to the incidence of colic in horses. In: Proc. 14th Equine Nutrition and Physiology Symposium. 242-247.

Belyea, R.L., F.A. Martz, and S. Bell. 1985. Storage and feeding losses of large round bales. J. Dairy Sci. 68: 3371-3375.

Bennett, G.A., T.C. Nelsen, and B.M. Miller. 1994. Enzyme-linked immunosorbent assay for detection of zearalenone in corn, wheat and pig feed: collaborative study. J. Assoc. Off. Anal. Chem. 77: 1500-1509.

Bondy, G. S. and J. J. Pestka. 2000. Immunomodulation by fungal toxins. J. Toxicol. Env. Health. B. 3:109-143.

Carson, M.S. and T.K. Smith. 1983. Effect of feeding alfalfa and refined plant fibers on the toxicity and metabolism of T-2 toxin in rats. J. Nutr. 113:304-313.

Casteel, S.W., G.E. Rottinghaus, G.C. Johnson, and D.T. Wicklow. 1995. Liver disease in cattle induced by consumption of moldy hay. Vet. Human Toxicol. 37: 248-251.

Chaouloff, F.D., D. Laude, D. Merino, B. Serrurrier, and J.L. Elghoji. 1986. Peripheral and central short-term effects of fusaric acid, a DBH inhibitor, on tryptophan and serotonin metabolism in the rat. J. Neural Transm. 65:219:232.

Chowdhury, S.R. and T.K. Smith. 2004. Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on performance and metabolism of laying hens. J. Anim. Sci. 83:1849-1856.

Clarke, A.F. 1993. Stable dust-threshold limiting values, exposure variables and host risk factors. Eq. Vet. J. 25: 172-174.

Clarke, A.F., T.M. Madelin, and R.J. Allpress. 1987. The relationship of air hygiene in stables to

lower airway disease and pharyngeal lymphoid hyperplasia in two groups of thoroughbred horses. Eq. Vet. J. 19: 524-530.

Collins, M. 1989. Conditioning effects and field variation in dry matter concentrations of alfalfa hay. Pages 1005-1006 in Proc. XVI Int. Grassland Congress. 1005-1006.

Corbaz, R., P.H. Gregory, and M.E. Lacey. 1963. Thermophilic and mesophilic actinomycetes in moldy hay. J. Gen. Microbiol. 32: 449-455.

Cote, L. M., V. R. Beasley, P. M. Bratich, S. P. Swanson, H. L. Shivaprasad, and W. B. Buck. 1985. Sex-related reduced weight gains in growing swine fed diets containing deoxynivalenol. J. Anim. Sci. 61:942-950.

Curtis, E.F., S.L. Raymond, and A.F. Clarke. 1996. Respirable dust and atmospheric ammonia levels in horse stalls with different ventilation rates and bedding. Int. J. Aerobiology. 12: 239-247.

Davis, N. D., J. W. Dickens, R. L. Freie, P. B. Hamilton, O. L. Shotwell, T. D. Wyllie, and J. F. Fulkerson. 1980. Protocols for surveys, sampling, post-collection handling, and analysis of grain samples involved in mycotoxin problems. J. Assoc. Off. Anal. Chem. 63:95-102.

D'Mello, J.P.F., C.M. Placinta, A.M.C. Macdonald. 1999. Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Anim. Feed Sci. Technol. 80:183-205.

Dong, W., J.E. Sell, and J.J. Pestka. 1991. Quantification assessment of mesangial immunoglobulin A (IgA) accumulation, elevated circulating IgA immune complexes, and hematuria during VT-induced IgA nephropathy. Fundam. Appl. Toxicol. 17:197-207.

Dyke, T.M., R.A. Sams, and K.W. Hinchcliff. 1998. Exercise-training-induced alterations in hepatic function in mares. J. Appl. Phy. 85:1442-1447.

Erlich, R. 1980. Interaction between environmental pollutants and respiratory infections. Environ. Health Perspect. 35: 89-100.

Eyre, P. 1972. Equine pulmonary emphysema: A bronchopulmonary mould allergy. Vet. Rec. 91: 134-140.

Flannigan, B., and J.D. Miller. 1995. Health Implications of Fungi in Indoor Environments. pp 3-28. Elsevier, Amsterdam.

Forgacs, J. 1972. Stachybotryotoxicosis, p. 95-128. In S. Kadis, A. Ciegler, and S.J. Ajl (ed.), Microbial toxins. Vol. VIII. Academic Press, Inc., New York.

Forsell, J. H., M. F. Witt, J. H. Tai, R. Jensen, and J. J. Pestka. 1986. Effects of 8-week exposure of the B6C3F1 mouse to dietary deoxynivalenol (vomitoxin) and zearalenone. Food Chem. Toxicol. 24:213-219.

Gimeno, A., and J.A. Quintanilla. 1983. Analytical and mycological study of a natural outbreak of zearalenone mycotoxicosis in horses. Pages 387-392 in Proc. Int. Symp. Mycotoxins. National Research Centre, Cairo, Egypt.

Greenhalgh, R., G.A. Neish and J.D. Miller. 1983. Deoxynivalenol, acetyldeoxynivalenol, and zearalenone formation by Canadian isolates of Fusarium graminearum on solid substrates. Appl. Environ. Microbiol. 46: 625.

Gregory, P.H., M.E. Lacey, G.N. Festenstein, and F.A. Skinner. 1963. Microbial and biochemical changes during the molding of hay. J. Gen. Microbiol. 33: 147-174.

Harrison, L.R., B.B. Colvin, J. Todd Greene, L. E. Newman, and J. R. Cole. 1990. Pulmonary edema and hydrothorax in swine produced by fumonisin B1 a toxic metabolite of Fusarium moniliforme. J. Vet. Diagn. Invest. 2:217-221.

Haschek, W.M., L.A. Gumprecht, G. Smith, M.E. Tumbleson, and P.D. Constable. 2001. Fumonisin toxicosis in swine: An overview of procine pulmonary edema and current perspectives. Eviron. Health Perspect. 109:251-257.

Hidaka, H. T., T. Nagatsu, K. Takeya, T. Takeuchi, H. Suda, K. Kojiri, M. Matsuzaki, and H. Umezawa. 1969. Fusaric acid, a hypotensive agent produced by fungi. J. Antibiot. 22:228-230.

Hintz, H.F. 1991. Moldy corn, megacolon and hyperlipidemia. Eq. Prac. 13: 23-24.

Horner, W.E., Helbling, A., Salvaggio, J.E. and S.B. Lehrer. 1995. Fungal Allergens. Clin. Microbiol. Rev. 8: 161-179.

Houpt, K.A.1990. Ingestive behavior. Eq. Prac. 6:319-337.

Houpt, K.A., and S.M. McDowell. 1993. Equine stereotypies. Comp. Cont. Edu. Prac. Vet. 15:1265-1272.

Huwig A., S. Freimund, O. Kappeli, and H. Dutler. 2001. Mycotoxin detoxification of animal feed by different adsorbents. Toxicol. Lett. 122:179-188.

James, L. J., and T. K. Smith. 1982. Effect of dietary alfalfa on zearalenone toxicity and metabolism in rats and swine. J. Anim. Sci. 55:110-118.

Johnson, P.J., S.W. Casteel, and N.T., Messer. 1997. Effect of feeding deoxynivalenol (vomitoxin)-contaminated barley to horses. J. Vet. Diagn. Invest. 9:219-221.

Johnson, D.G., D.E. Otterby, R.G. Lundquist, J.A. True, F.A. Benson, R.E. Smith, and J. Lacey. 1975. Potential hazards to animals and man from microorganisms in fodders and grain. Trans. Brit. Myco. Soc. 65: 171-184.

Johnson, D.G., D.E. Otterby, R.G. Lundquist, J.A. True, F.A. Benson, R.E. Smith, L.K. Lindor, and R.C. Stommes. 1984. Yield and quality of alfalfa as affected by harvesting and storage methods. J. Dairy Sci. 67: 2475-2480.

Juhasz, J., P. Nagy, G. Huszenicza, G. Szigeti, J. Reiczigel, and M. Kulesar. 1997. Long term exposure to T-2 fusarium mycotoxin fails to ulter luteal function, follicular activity and embyro recovery in mares. Equine Vet. J. Suppl. 25:17-21.

Juhasz, J., P. Nagy, M. Kulesar, G. Szigeti, J. Reiczigel, and G. Huszenicza, 2001.Effect of low-dose zearolenone exposure on luteal function, follicular activity and uterine oedema in cycling mares. Acta Veterinaria Hungarica. 49(2):211-222.

Kalleta, K. and E. Ettala. 1981. The oestrogenic Fusarium toxin (zearalenone) in hay as a cause of early abortions in the cow. Nord. Vet. Med. 36:305-309.

Kotimaa, M.J., K.H. Husman, E.O. Terho, and M.H. Mustonen. 1984. Airborne molds and actinomycetes in the work environment of farmer's lung patients in Finland. Scand. J Work Environ. Health. 10:115-119.

Kuehl, R. O. 2000. Design of experiments: Statistical Principles of Research Design and Analysis. 2nd ed. Duxbury Press. Belmont, CA.

Lacey, J. 1975. Potential hazards to animals and man from microorganisms in fodders and grain. Transactions of the British Mycology Society 65: 171-184.

Leathwood, D. 1987. Tryptophan availiability and serotonin synthesis. Proc. Nutr. Soc. 46:143-156.

Lindner, A., M. Sobotta, M. and H.H.L., Sasse. 2001. Predictability of blood lactate concentration after exercise in horses working at speeds that were guided by results of a exercise stress test. Part 2: Relation between heart rate during exercise and blood lactate concentrations after exercise. Pferdeheilkunde. 17: 241-246.

Mancini, G., A.O. Carbonara, and J.F. Heremans. 1965. Immunochemical quantification of antigens by single radial immunodiffusion. Immunochemistry. 2:235-254.

Matsui, Y., and M. Watanabe. 1988. Quantitative analysis of fusaric acid in the cultural filtrate and soybean plants inoculated with *Fusarium oxysporum* var. redolens. J. Rakuno Gakuen Univ. Nat. Sci. 13:159-167.

McCall, C.A. 1993. Wood chewing by horses. Eq. Prac. 15: 35-36.

McGorum, B.C., P.M. Dixon, and R.E. Halliwell. 1993. Responses of horses affected with

chronic obstructive pulmonary disease to inhalation challenges with mold antigens. Eq. Vet. J. 25: 261-267.

Meloche, J. L., and T. K. Smith. 1995. Altered tissue amino acid metabolism in acute T-2 toxicosis. Proc. Soc. Exp. Biol. Med. 210:260-266.

Northolt, M.D., C.A.H. Verhulsdonk, and P.S.S. Soentoro. 1976. Effect of water activity and temperature on aflatoxin production by Aspergillus parasiticus. J. Milk Food Technol. 39:170-174.

NRC. 1989. Pages 43–44 in Nutrient Requirements of Horses. 5th ed. Natl. Acad. Press, Washington, DC.

Oberdörster, G. 1995. Lung particle overload: Implications for occupational exposures to particles. Reg. Toxicol. Pharm. 27: 123-135.

Palmgren, M.S. and L.S. Lee. 1986. Separation of mycotoxin-containing sources in grain dust and determination of their mycotoxin potential. Environ. Health Perspect. 66:105-108.

Pestka, J.J., M.A. Moorman, and R.L. Warner. 1989. Dysregulation of IgA production and IgA nephropathy induced by trichothecene vomitoxin. Food Chem. Toxicol. 27:361-368.

Pond, K.R., D.K. Dugan, and D.T. Barnett. 1995. Grazing behavior of mares and steers on orhardgrass and mares on common bernuda grass. Pages 236-237 in Proc. 14th Eq. Nutr. Physiol. Soc.

Porter, J. K., C. W. Bacon, E. M. Wray, and W. M. Hagler Jr. 1995. Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. Nat. Toxins. 3:91-100.

Porter, J.K., K.A. Voss, C.W. Bacon, and W.P. Norred. 1990. Effects of Fusarium moniliforme and corn associated with equine leukoencephalomalacia on rat neurotransmitters and metabolites. Proc. Soc. Exper. Biol. Med. 194:265-269.

Porter, J.K., K.A. Voss, W. J. Chamberlain , C.W. Bacon, and W.P. Norred. 1993. Neurotransmitters in rats fed fumonisin B1. Proc. Soc. Exper. Biol. Med. 202:360-364.

Prelusky, D. B. 1993. The effect of low-level deoxynivalenol on neurotransmitter levels measured in pig cerebral spinal fluid. J. Environ. Sci. Health. B. 28:731-761.

Raju, M. V. L. N., and G. Devegowda. 2000. Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Br. Poultry Sci. 41:640-650.

Ramos, A. J., J. Fink-Gremmels, and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins by means of non-nutritive adsorbent compounds. J. Food Protect. 59:631-641.

Raymond, S.L., E.F. Curtis, L.M. Winfield, and A.F. Clarke. 1997. A comparison of respirable particles associated with various forage products for horses. Eq. Prac. 19: 23-26.

Raymond, S.L., T.K. Smith, and H.V.L.N. Swamy. 2003. Effects of feeding a blend of grains naturally-contaminated with *Fusarium* mycotoxins on feed intake, serum chemistry and hematology of horses. J. Anim. Sci. 81:2123-2130.

Rossi-Fanelli, F. and C. Cangiano. 1991. Increased availability of tryptophan in brain as common pathologic mechanism of anorexia associated with different diseases. Nutrition 7:364.

Ross, P.F., L.G. Rice, R.D. Plattner, G.D. Osweiler, T.M. Wilson, D.L. Owens, H.A. Nelson, J.L. Richard. 1991. Concentrations of fumonisin B1 in feeds associated with animal health problems. Mycopathologia. 114:129-135.

Ross, P.F., L.G. Rice, G.D. Osweiller, P.E. Nelson, J.L. Richard, and T.M. Wilson. 1992. A review and update of animal toxicosis associated with fumonisin-contaminated feeds and production of fumonisins by *Fusarium* isolates. Mycopathologia. 117:109-114. Ross, P.F., P.E.

Nelson, D.L. L.G. Owens, H.A. Rice, T.M. Nelson, and T.M. Wilson. 1994. Fumonisin B2 in cultured Fusarium proliferatum, M-6104, causes equine leukoencephalomalacia. J. Vet. Diagn. Invest. 6:263-265.

Rotter, B.A., B.K. Thompson, M. Lessard, H.L. Trenholm, and H. Tryponas. 1994. Influence of low-level exposure to Fusarium mycotoxins on selected immunological and hematological parameters in young swine. Fundam. Appl. Toxicol. 23:117-124.

Russel, B.S. 1974. Mycology Guidebook, Washington Press, Seattle, W.A.

Schindler, A.F. 1977. Temperature limits for production of aflatoxin by 25 isolates of Aspergillus flavus and Aspergillus parasiticus. J. Food Protect. 40:39-40.

Sklan, D., E. Klipper, and A. Friedman. 2001. The effect of chronic feeding of DAS, T-2 toxin and aflatoxin on performance, health and antibody production in chicks. J. Appl. Poultry Research 10:79-85.

Smith, G.W., P.D. Constable, J.H. Foreman, R.M. Eppley, A.L. Waggoner, M.E. Tumbleson, and W.M. Haschek. 2002. Cardiovascular changes associated with intravenous administration of fumonisin B1 in horses. Am. J. Vet. Res. 63:538-545.

Smith, T.K., and I.R. Seddon. 1998. Toxicological synergism between Fusarium mycotoxins in

feeds. In: *Biotechnology in the feed industry.* (ed. T.P. Lyons and K.A. Jacques) pp-257-269. Nottingham Univ. Press, Nottingham.

Smith, J.E. and R.S. Henderson. 1991. Mycotoxins and animal foods. CRC press, Inc., Boca Raton, Florida.

Smith, T.K., E.G. McMillan, and J.B. Costillo. 1997. Effect of feeding blends of Fusarium mycotoxin-contaminated grains containing deoxynivalinol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75: 2184-2191.

Smith, T. K., and E. J. MacDonald. 1991. Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. J. Anim. Sci. 69:2044-2049.

Smith, T. K., E. G. McMillan, and J. B. Castillo. 1997. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75:2184-2191.

Smith, T. K., and M. G. Sousadias. 1993. Fusaric acid content of swine feedstuffs. J. Agric. Food Chem. 41:2296-2298.

Sobotta, M., A. Lindner, and H.H.L., Sasse. 2001. Predictability of blood lactate concentration after exercise in horses working at speeds that were guided by results of a exercise stress test. Part 1:Lactate guided speed. Pferdeheilkunde. 17: 42-46.

Speijers, G.J.A. and M.H.M. Speijers. 2004. Combined toxic effects of mycotoxins. Toxicol. Lett. 153:91-98.

Swamy, H. V. L. N., and G. Devegowda. 1998. Ability of mycosorb in counteracting aflatoxicosis in commercial broilers. Indian J. Poult. Sci. 33:273-278.

Swamy, H. V. L. N., T. K. Smith, P.F. Cotter, H.J. Boermans, and A.E. Sefton. 2002a. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on production and metabolism in broilers. Poult. Sci. 81: 966-975.

Swamy, H. V. L. N., T. K. Smith, E.J. MacDonald, H.J. Boermans, and E.J. Squires. 2002b. Effects of feeding a blend of grains naturally contaminated with *Fusarium* mycotoxins on swine performance, brain regional neurochemistry and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80: 3257-3267.

Swamy, H. V. L. N., T. K. Smith, E.J. MacDonald, N.A. Karrow, B. Woodward and H.J. Boermans. 2003. Effects of feeding a blend of grains naturally-contaminated with *Fusarium* mycotoxins on growth and immunological parameters of starter pigs, and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 81:2792-2803.

Swamy, H. V. L. N., T. K. Smith, E.J. MacDonald, N.A. Karrow, and H.J. Boermans. 2004a. Effects of feeding blends of grains naturally-contaminated with *Fusarium* mycotoxins on growth and immune response of broiler chickens. Poult. Sci. 83:533-543.

Swamy, H.V.L.N., T.K. Smith and E.J. MacDonald. 2004b. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on brain regional neurochemistry of starter pigs and broiler chickens. J. Anim. Sci. 82:2131–2139.

Tarter, E.J., J.P. Hanchay, and P.M. Scott. 1984. Improved liquid chromatographic method for determination of aflatoxins in peanut butter and other commodities. J. Assoc. Off. Anal. Chem. 67:597-600.

Thomson, J.R., and E.A. McPherson. 1984. Effects of environmental control on pulmonary function of horses affected with chronic obstructive pulmonary disease. Eq. Vet. J. 16: 35-38.

Trenholm, H. L., B. C. Foster, L. L. Charmley, B. K. Thompson, K. E. Hartin, R. W. Coppock, and M. A. Albassam. 1994. Effects of feeding diets containing *Fusarium* (naturally) contaminated wheat or pure deoxynivalenol (DON) in growing pigs. Can. J. Anim. Sci. 74:361-369.

Van der Westhuizen, L., G.S. Shephard, and D.J. van Schalkwyk. 2001. The effect of a single gavage dose of fumonisin B1 on the sphinganine and sphingosine levels in vervet monkeys. Toxicon. 39:273-281.

Venturini M.C., M.A. Quiroga, M.A. Risso, C. Di Lorenzo, Y. Omata, L. Venturini and H. Godoy. Mycotoxin T_2 and Aflatoxin B_1 as immunosuppresors in mice chronically infected with *Toxoplasma gondii*. J. Comp. Path. 115: 229-237, 1996.

Wannemacher, R.W., D.L. Bunner, and H.A. Neufeld. 1991. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. *In: Mycotoxins and Animal Feeds* (J.E. Smith and R.S. Henderson, eds) CRC Press, Boca Raton, FL.

Wannemacher, R.W. and R.E. Dinterman. 1983. Plasma amino acid changes in guinea pigs injected with T-2 mycotoxin. Fed. Proc. 42:625 (abstract).

Willoughby, R.A., G.L. Ecker, S.L. McKee, and L.J. Riddolls. Use of scintigraphy for the determination of muco-ciliary clearance rates in normal, sedated, diseased and exercised horses. Can. J. Vet. Res. 55: 315-320.

Wood, G.E. 1992. Mycotoxins in foods and feeds in the United States. J. Anim. Sci. 70:3941-3949.

Zeyner, A., U. Fischer, and A. Lindner. 2002. Distinct weight loss and elevated hepatic enzymes in horses caused by straw contaminated with Deoxynivalenol (case report). Proceedings of the Comparative Nutrition Society, Antwerp,, Belgium.

Appendix I. Effect of feeding concentrates naturally contaminated with *Fusarium* mycotoxins on d 7, 14 and 21 complete and differential white blood cell counts.

Dietary Group	WBC[a]	SNC[b]	LC[c]	MC[d]	EC[e]
			Day 7		
Control[f]	8.22[g, h]	3.65	3.76	0.21	0.49
Mycotoxin[i]	8.28	4.05	3.64	0.23	0.30
0.2% GM[j]	8.17	3.72	3.94	0.26	0.25
SEM	0.12	0.18	0.15	0.05	0.05
			Day 14		
Control	8.64	3.99	4.25	0.23	0.13
Mycotoxin	8.44	4.03	3.80	0.33	0.25
0.2% GM	8.72	4.20	3.76	0.26	0.39
SEM	0.15	0.12	0.20	0.03	0.04
			Day 21		
Control	8.32	4.30	3.66	0.23	0.22
Mycotoxin	8.06	4.86	2.88	0.18	0.17
0.2% GM	8.83	5.22	3.09	0.25	0.41
SEM	0.32	0.19	0.16	0.33	0.12

[a]White blood cell count, 10^9/L.

[b]Segmented neutrophil count, 10^9/L.

[c]Lymphocyte count, 10^9/L.

[d]Monocyte count, 10^9/L.

[e]Eosinophil count, 10^9/L.

[f]Concentrate with control corn and wheat.

[g]No significant effect of diet on differential leukocyte count was observed ($P > 0.05$).

[h]Values are least-square means; n = 6 horses.

[i]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[j]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

Appendix II. Effect of feeding concentrates naturally contaminated with *Fusarium* mycotoxins on d 7, 14 and 21 hematological parameters.

Dietary Group	RBC[a]	Hb[b]	HCT[c]	MCH[d]	Platelets[e]
			Day 7		
Control[f]	8.28[g, h]	139.80	0.36	17.00	169.50
Mycotoxin[i]	8.10	136.40	0.35	17.00	155.40
0.2% GM[j]	8.12	138.17	0.36	17.17	173.80
SEM	0.15	1.87	0.01	0.09	10.50
			Day 14		
Control	8.56	145.20	0.38	17.20	140.80
Mycotoxin	8.30	139.60	0.37	16.80	137.00
0.2% GM	8.47	143.00	0.38	17.00	146.50
SEM	0.11	1.63	0.00	0.08	3.47
			Day 21		
Control	8.40	142.20	0.37	17.00	174.75
Mycotoxin	7.84	133.60	0.35	17.00	150.20
0.2% GM	8.08	138.17	0.36	17.00	155.33
SEM	0.15	2.41	0.01	0.00	15.52

[a]Red blood cell count, 10^9/L.

[b]Hemoglobin, g/L.

[c]Hematocrit, L/L.

[d]Mean corpuscular hemoglobin, pg.

[e]Platelets, 10^9/L.

[f]Concentrate with control corn and wheat.

[g]No significant effect of diet on hematological parameters were observed ($P > 0.05$).

[h]Values are least-square means; n = 6 horses.

[i]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[j]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

Appendix III. Effect of feeding concentrates naturally contaminated with *Fusarium* mycotoxins on d 7, 14 and 21 selected serum metabolites.

Dietary Group	GGT[a]	AST[b]	CK[c]	GLDH[d]	TB[e]	AP[f]
			Day 7			
Control[g]	56.17[h, i]	421.25	268.63	17.83	20.46	217.33
Mycotoxin[j]	42.54	361.04	141.29	8.96	21.96	213.00
0.2% GM[k]	40.67	345.83	174.33	5.83	18.83	195.17
SEM	6.47	18.19	23.18	4.53	0.77	7.23
			Day 14			
Control	74.88	541.46	348.50	70.58	21.42	236.29
Mycotoxin	53.21	382.83	124.75	59.08	27.38	229.33
0.2% GM	47.67	453.33	415.00	54.83	22.33	212.50
SEM	10.55	39.21	90.26	5.83	1.40	12.63
			Day 21			
Control	110.45	697.54	193.13	4.74	53.08	294.25
Mycotoxin	64.04	340.17	194.71	4.74	34.92	223.42
0.2% GM	60.50	483.67	170.67	3.87	31.50	218.33
SEM	17.65	64.67	8.71	2.37	8.09	29.82

[a]Gamma-glutamyltransferase, U/L

[b]Asparate Aminotransferase, U/L.

[c]Creatine Kinase, U/L.

[d]Glutamic Dehydrogenase, U/L.

[e]Total bilirubin, umol/L.

[f]Alkaline Phosphatase , U/L.

[g]Concentrate with control corn and wheat.

[h]No significant effect of diet on serum metabolites were observed ($P > 0.05$).

[i]Values are least-square means; n = 6 horses.

[j]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[k]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

Appendix IV. Effect of feeding concentrate naturally contaminated with *Fusarium* mycotoxins on selected parameters during a time to fatigue treadmill step test.

Dietary group	Resting heart rate[a]	Starting heart rate	Time of fatigue heart rate	5 min recovery heart rate	10 min recovery heart rate	Time to fatigue, minutes
Control[b]	40[c, d]	125	173	87	71	12.32
Mycotoxin[e]	39	135	175	82	75	11.14
0.2% GM[f]	35	140	155	79	72	9.94
SEM	1.39	4.33	4.80	2.68	1.55	0.64

[a]Beats per minute.

[b]Concentrate with control corn and wheat.

[c] No significant effects of diet on heart rate or time to fatigue were observed ($P > 0.05$).

[d] Values are least-square means; n = 6 horses.

[e]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[f]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

Appendix V. Effect of feeding concentrate naturally contaminated with *Fusarium* mycotoxins on selected blood parameters during a time to fatigue treadmill step test.

Dietary group	WBC[a]	LC[b]	RBC[c]	HB[d]	HCT[e]
			Resting		
Control[f]	8.32[g, h]	3.66	8.40	142.20	0.37
Mycotoxin[i]	8.06	2.88	7.84	133.60	0.35
0.2% GM[j]	8.83	2.70	8.08	138.17	0.36
SEM	0.34	0.20	0.11	1.90	0.01
			Time of Fatigue		
Control	11.56	4.77	11.41	195.63	0.52
Mycotoxin	11.56	4.42	11.02	188.40	0.49
0.2% GM	11.88	4.31	10.77	185.50	0.48
SEM	0.38	0.33	0.08	1.64	0.01
			10 minute recovery		
Control	10.40	4.76	9.66	164.40	0.43
Mycotoxin	10.56	4.02	9.08	156.20	0.40
0.2% GM	11.18	4.13	9.13	158.00	0.40
SEM	0.18	0.17	0.09	1.49	0.00

[a]White blood cell count, 10^9/L.

[b] Lymphocyte count, 10^9/L.

[c]Red blood cell count, 10^9/L.

[d]Hemoglobin, g/L.

[e]Hematocrit, L/L.

[f]Concentrate with control corn and wheat.

[g]No significant effect of diet on blood parameters were observed ($P > 0.05$).

[h]Values are least-square means; n = 6 horses.

[i]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[j]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.

Appendix VI. Effect of feeding concentrate naturally contaminated with *Fusarium* mycotoxins on serum lactate levels during a time to fatigue treadmill step test.

Dietary group	Resting lactate[a]	Time of fatigue lactate	5 min recovery lacate	10 min recovery lactate	Peak lactate	Time to peak lactate, minutes
Control[b]	1.16[c, d]	3.78	2.32	2.04	3.78	12.65
Mycotoxin[e]	1.18	3.74	2.10	1.64	3.74	11.70
0.2% GM[f]	1.15	3.15	1.97	1.63	3.25	9.72
SEM	0.05	0.08	0.08	0.05	0.08	0.66

[a]Lactate, mmol/L.

[b]Concentrate with control corn and wheat.

[c]No significant effect of diet on lactate or time to peak lactate were observed ($P > 0.05$).

[d]Values are least-square means; n = 6 horses.

[e]Mycotoxin-contaminated concentrate containing 35.8% contaminated wheat and 53.6% contaminated corn.

[f]Mycotoxin-contaminated concentrate supplemented with 0.2% glucomannan polymer.